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ISBN 0-315-55497-5

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THE UNIVERSITY OF ALBERTA

"The morphology, endocrine mediation of development and correlation with reproductive activity of the anal-fin-fold, a secondary sexual character in male *Ophioblennius atlanticus* (Pisces: Blenniidae)"

by

Baljinder Singh (C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

Department of Zoology

Edmonton, Alberta

Fall, 1989

THE UNIVERSITY OF ALBERTA

RELEASE FORM

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DEGREE FOR WHICH THESIS WAS PRESENTED Master of Science

YEAR THIS DEGREE GRANTED Fall, 1989

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The undersigned certify that they have read, and recommend, to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The morphology, endocrine mediation of development and correlation with reproductive activity of the anal-fin-fold, a secondary sexual character in male *Ophioblennius atlanticus* (Pisces: Blenniidae)" submitted by Baljinder Singh in partial fulfilment of the requirements for the degree of Master of Science.

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Date June 15, 1989

ABSTRACT

This study has focused on the structure of anal-fin modifications around the urogenital region of sexually mature male Ophioblennius atlanticus in Barbados, West Indies. Such structures, referred to as "anal-fin-folds" or "AFFs", are the most obvious feature of sexual dimorphism in the species.

Each AFF is complex in structure, consisting of both dermal and epidermal components which completely enclose one of two anal-fin spines. AFF epidermal tissue is stratified and in its most developed form is composed of basic and superficial epithelial cells as well as two unicellular gland types: mucous and serous goblet cells. Neutral and sulphated glycoproteins are a major constituent of mucous cell secretion whereas serous cells primarily secrete a proteinaceous substance. Ultrastructural examination of serous goblet cells revealed granules which display a previously unreported concentric-patterned substructure.

Ophioblennius atlanticus has an extended breeding season with a lunar periodicity. AFF development also displays a clear lunar periodicity with maximal size occurring at full moon, the time of peak spawning activity. AFF developmental changes mainly occur within the epidermis and involve an overall thickening of this tissue and an increase in size and secretory activity of serous and mucous goblet cells.

Strong evidence is provided for the involvement of androgens in AFF development, with both dermal and epidermal tissues being affected. Within the epidermis, serous goblet

cells were apparently most sensitive to 11-ketotestosterone treatment, showing developmental changes similar to those observed in field samples collected around full moon.

The basic morphology of a large testicular gland, peculiar to blenniids, is also described. Preliminary findings of correlative changes in the AFF and testicular gland are presented.

Finally, the possible functions of the AFF are discussed.

ACKNOWLEDGEMENTS

Sincerest thanks are extended to both of my supervisors, Dr. Ralph Nursall and Dr. Norm Stacey. Their support, instruction, advice and constructive criticism were instrumental in the completion of this thesis. Thanks also to my supervisory committee members, Dr. Joe Nelson and Dr. George Evans, who, in the process of enduring four arduous committee meetings, provided much by way of valuable feedback.

I am indebted to Randy Mandryk and Ron Koss for their much valued technical instruction, expertise and assistance in the histological and electron microscopical studies respectively. Dr. John Buckland-Nicks was also very helpful with the EM study.

I am grateful to Dr. Wayne Hunte, director of Bellairs research institute in Barbados, for the use of its facilities. Thanks also to the staff of Bellairs, who were always helpful (albeit sometimes for a price !). I would also like to acknowledge various water-sports small business operators in the St. James area in Barbados, whose best efforts to impede my fish collecting and indeed, hasten my demise, failed dismally--better luck next time, guys !

Thanks to Shyamala Nagendran and Lorne Leclair for guiding me through innumerable computer-related nightmares. I appreciate the efforts of Wolfgang Janzen and Jim Van Es in translating several previously indecipherable German publications. Various others in the department have been most

supportive throughout my stay in Edmonton, including Maggie Haag and all the Zoology office staff---many thanks.

Finally, for their aid, encouragement and camaraderie, a word of appreciation to the real workhorses of the department, my fellow graduate students.....

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I. INTRODUCTION

Members of the Blenniidae share a number of prominent morphological characteristics, such as cirri on the head and nape, head crests, and epidermal glands in median fins as well as anal fin modifications around the urogenital region. Some of these features have been described as secondary sexual characters (Zander, 1975; Papaconstantinou, 1979; Patzner and Seiwald, 1987) and reported to vary seasonally in size and development (Smith, 1974; Papaconstantinou, 1979; Patzner and Seiwald, 1987). However, there are no detailed studies documenting how these structures might change with the reproductive cycles.

This study has focused on the structures associated with the first and second anal fin spines of male redlip blennies, Ophioblennius atlanticus macclurei(Silvester). There is considerable variation in morphology of these anal fin modifications among the Blenniidae (Porter, 1979) and consequently the nomenclature is inconsistent. These structures have been referred to as anal-fin-pads (Patzner and Seiwald, 1987; Smith, 1974), anal-fin-bulbs (Zander, 1975; Papaconstantinou, 1979), anal-fin-knobs (Peterson, 1984) and anal-fin-folds (AFF: Eggert, 1931; Kotrschal and Goldschmid, 1983). The latter nomenclature will be used in this thesis.

The AFF of O. atlanticus form the most obvious feature of sexual dimorphism in the species. Other secondary sexual features common to Mediterranean species (Zander, 1975;

Papaconstantinou, 1979) are not apparent in this species. This is the first study of the AFF in O. atlanticus.

The redlip blenny is a small (maximum standard length = 100 mm), herbivorous fish commonly occurring in shallow-water fringing reef communities. It is found in the western central Atlantic from North Carolina and Bermuda to Venezuela (Springer, 1962). Both males and females reach maturity at 45.0 mm standard length and hold permanent territories on reefs in shallow water (Nursall, 1977). Ovulated females seek out nesting males, communicate their readiness to spawn and return to their territories after spawning (Marraro and Nursall, 1983). As is typical of blenniids (Blumer, 1979), the male redlip blenny cares for and defends the adhesive eggs in a prepared nest crevice within his territory (Böhlke and Chaplin, 1968; Marraro and Nursall, 1983).

Spawning of O. atlanticus occurs all year but is known to be influenced by the lunar cycle (Marraro and Nursall, 1983; Côté, 1987). Côté (1987) found that a distinct peak in spawning occurs three days before full moon (FM) but that the reproductive period (as assessed by counting the number of egg batches in 44 nests) extended over two weeks every month, ending abruptly several days after FM. Two previous studies (Marraro, 1978; Labelle, 1982) on the population dynamics and reproductive behaviour of redlip blennies in Barbados also presented gonado-somatic index (GSI) data. However, Marraro (1978) collected male and female GSI data only on a monthly basis using small samples ($n<7$). Labelle (1982) concentrated

only on female GSIs, collecting between six and ten females randomly every four days over five months. Since neither of these studies offered a careful examination of GSI changes in relation to the lunar cycle, such information is provided here.

Analysis of GSI in male blenniids is complicated by the fact that their testes contain both seminiferous and glandular components (Eggert, 1931; Chieffi and Botte, 1964; Blüm, 1972; Patzner and Seiwald, 1987). The testicular gland is interposed between the seminiferous tissue and the efferent duct so that sperm have to pass through it to reach the vas deferens. This gland shows considerable variation morphologically among species of **Blennius**. Courrier (1922) thought the gland to have an endocrine function. Chieffi and Botte (1964) detected 3-β-hydroxysteroid-dehydrogenase positive cells in the gland of some blenniids. Blüm (1972) reported the testicular gland of **B. sphinx** to be an LH-dependent lipid body. The most complete study to date has been on **B. pavo** by Seiwald and Patzner (1987). Their histological, fine structural and histochemical investigations showed that seasonal variations of the gland cells are closely related to the reproductive cycle. They demonstrated that the gland is multi-functional with the main functions being the storage of lipids, the synthesis of steroids, having lysosomal activity similar to rat epididymis, and producing secretions which contribute to the seminal fluid. My preliminary histological and histochemical

investigations indicate a number of morphological similarities between the testicular gland of B. pavo and O. atlanticus. A similar gland is found in gobiids, although in that group the efferent duct is situated between the gland and the seminiferous portion so that sperm do not pass through the gland. Colombo (1979) found that in Gobius jozo, the gland is the site of intensive steroid production, especially production of steroid glucuronides proposed to function as reproductive pheromones.

The main objectives of the present study were to describe the structure of the AFF in O. atlanticus and to investigate its temporal relationship with the lunar reproductive cycle. An 18 week sampling study was conducted through four and a half months to determine how spawning activity (as assessed by gonadal changes) is related to the lunar cycle. This, together with histological and histochemical investigations, provided information on how changes in the size, development and cytochemical features of the AFF relate to the lunar spawning cycle. The epithelial component of the AFF was examined at the ultrastructural level by electron microscopy. The effects of androgens on the AFF were also studied. Finally, a preliminary examination of the correlative changes in the AFF and the testicular gland was undertaken.

The results show that maximal AFF development occurs at peak periods of spawning, thereby suggesting this structure plays some as yet unknown reproductive function in male O. atlanticus.

II. DESCRIPTION OF THE SAMPLING AREA

Field work was conducted between May and December 1987 at the Bellairs Research Institute of McGill University in St. James, Barbados (59° 37' W ; 13° 04' N).

Meteorological and oceanographic conditions around Barbados are fairly constant. Salinity in surface layers fluctuates from 33.5 °/oo (September - October) to 36.5 °/oo (February - March) while surface sea-temperatures range from 26.0°C in early winter to 29.5°C in late summer (Sander and Steven, 1973). The tidal rhythm is semidiurnal. Lewis (1960) estimated the mean tidal amplitude at 70 cm and the tidal range at 110 cm.

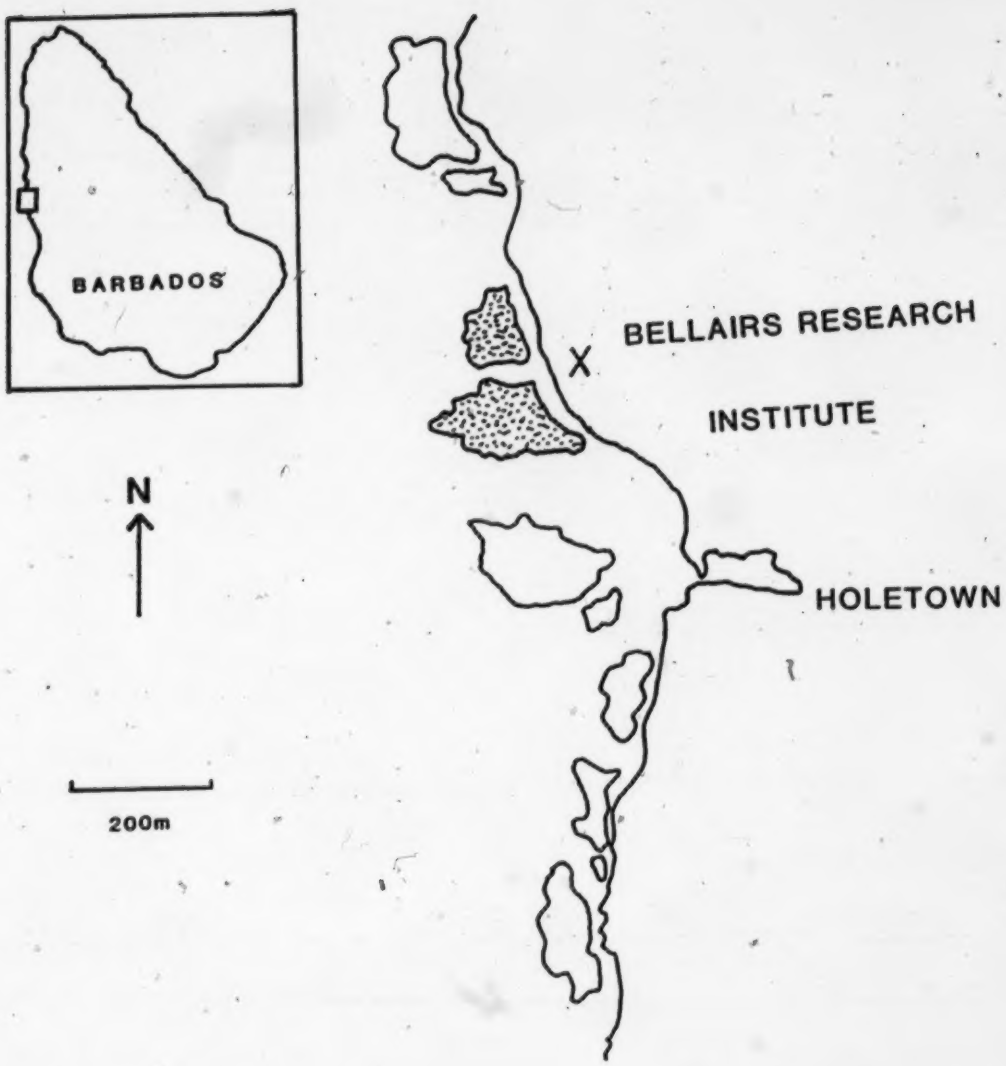
All fish samples were collected from 2 nearshore fringing reefs adjacent to the Bellairs Research Institute (Fig. 1). These reefs together were about 250 m long and 150 m wide with a depth varying between 1 and 8 m.

The topography and coral communities along the St. James coast of Barbados have been described previously (Lewis, 1960; Marraro, 1978). Three zones of inshore fringing reefs are recognized : Reef Flat Zone, Reef Crest Zone and the Seaward Slope. The Reef Flat Zone is a wide area (10 - 70 m) of flat reef rock that lies between the shore and the area of actively growing coral.

The Reef Crest Zone is the climax zone of the living reef. The basic features of this zone include spurs or ridges which project out toward the sea. Irregular grooves run between the spurs. The spurs lie perpendicular to the shore

FIGURE 1.

Geographic location of sampling site. The two reefs from which all fish were collected are stippled.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and have an irregular outline. The surface of the zone is characterized by dead coral rock which is secondarily encrusted with two coral species, **Porites porites** and **P. astreoides**. The dominant corals and chief structural elements of this zone are **Montastrea annularis** and **Siderastrea siderea**. Also abundant in this zone are **Millepora** sp. and **Montastrea cavernosa.**

At the outer limit of the Reef Crest Zone there are massive boulders which are separated by sand valleys. These ridges and valleys are continuous with the Reef Crest Zone. This area is characterized by a gradual slope of the reef to a depth of 6 to 8 m and is termed the Seaward Slope. The outer limit of the Seaward Slope is represented by low ridges which appear as outcrops (1 - 3 m high) from a sand bottom. The valleys are less regular and tend to divide and form large open sand patches. The dominant coral of the Seaward Slope is **Montastrea annularis.**

Previous population censuses (Nursall, 1981; Marraro, 1982; Côté, personal communication) indicate high densities of blennies in these fringing reefs with means of 1.9/m² (Nursall, 1981) and 1.67/m² (Marraro, 1982) being reported. **O. atlanticus** is most abundant in the Reef Crest Zone, occasionally found in the Reef Flat Zone and the inner areas of the Seaward Slope, and rarely found at the outer limits of the Seaward Slope. Most of the sampling was done in the Reef Crest Zone.

III. MATERIALS AND METHODS

A. Sampling procedure:

Males were sampled once a week from May 13, 1987 to September 14, 1987. Each weekly sampling day was chosen to coincide with the day of new moon (NM), full moon (FM) and the intervening quarter moon phases. Weekly sampling for females occurred two days after male sampling and ran from June 11, 1987 to September 9, 1987.

On each collection day, 15 sexually mature specimens (about 80 mm total length and 60 mm standard length) were collected using SCUBA. The fish were caught using open-ended, 15 cm, transparent plexiglass tubes of varying diameters, with clear plastic bags closing off one end. When approached, males often retreated into their nests or crevices. These would then be collected by first placing the open end of the plexiglass tube over the crevice entrance (or exit) and then probing the crevice with a length of wire to induce the blenny to enter the plastic bag. Although females rarely used crevices, they could be cornered and caught using a technique similar to that described above. From 6 to 8 hours were required to fill each sampling day's quota.

The captured specimens were maintained overnight indoors in glass aquaria with continuously flowing filtered, aerated sea-water (28°C). Morphometric measurements were taken the following day.

B. Morphometric investigation :

The blennies were sacrificed by anesthetization in 2-phenoxyethanol (0.05% in sea-water, Syndel). Standard length (SL) and total length (TL) measurements were taken to ± 0.1 mm with vernier calipers. Fish were blotted dry and weighed (± 0.1g) using a Mettler PE600 balance.

The gonads were removed and weighed on a Mettler HL52 analytical balance to ± 0.01 mg. The gonado-somatic Index [GSI = gonad weight in g/body weight in g x 100] for each fish was determined.

Only the anterior and more prominent of the two AFFs (Fig. 2, 3) was examined, as the second AFF was often too small to be measured accurately. To facilitate subsequent measurements, the AFFs were then excised by cutting them off at the base of the first anal spine with iridectomy scissors. The area occupied by an AFF was estimated by placing the AFF (oriented as in Fig. 3b) on a square grid (1 mm divisions) which was then examined under the dissecting microscope. An AFF-Area Index [AAI = AFF area in mm^2 /SL in mm x 100] was calculated for each male specimen. The AFFs were weighed to the nearest 0.01 mg. The AFF-Weight Index [AWI = AFF weight in g/ total weight in g x 100] was calculated for each male specimen.

FIGURE 2

Location of anal-fin-folds (A) in a male red-lip blenny.

B Anus

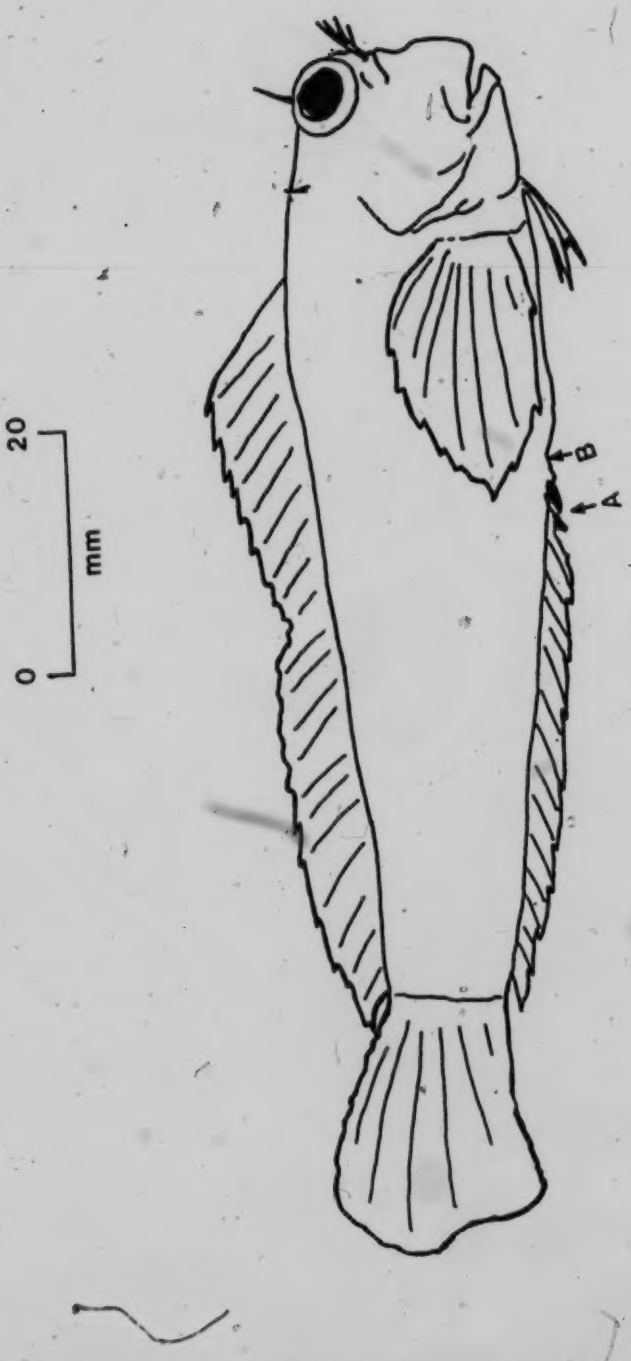
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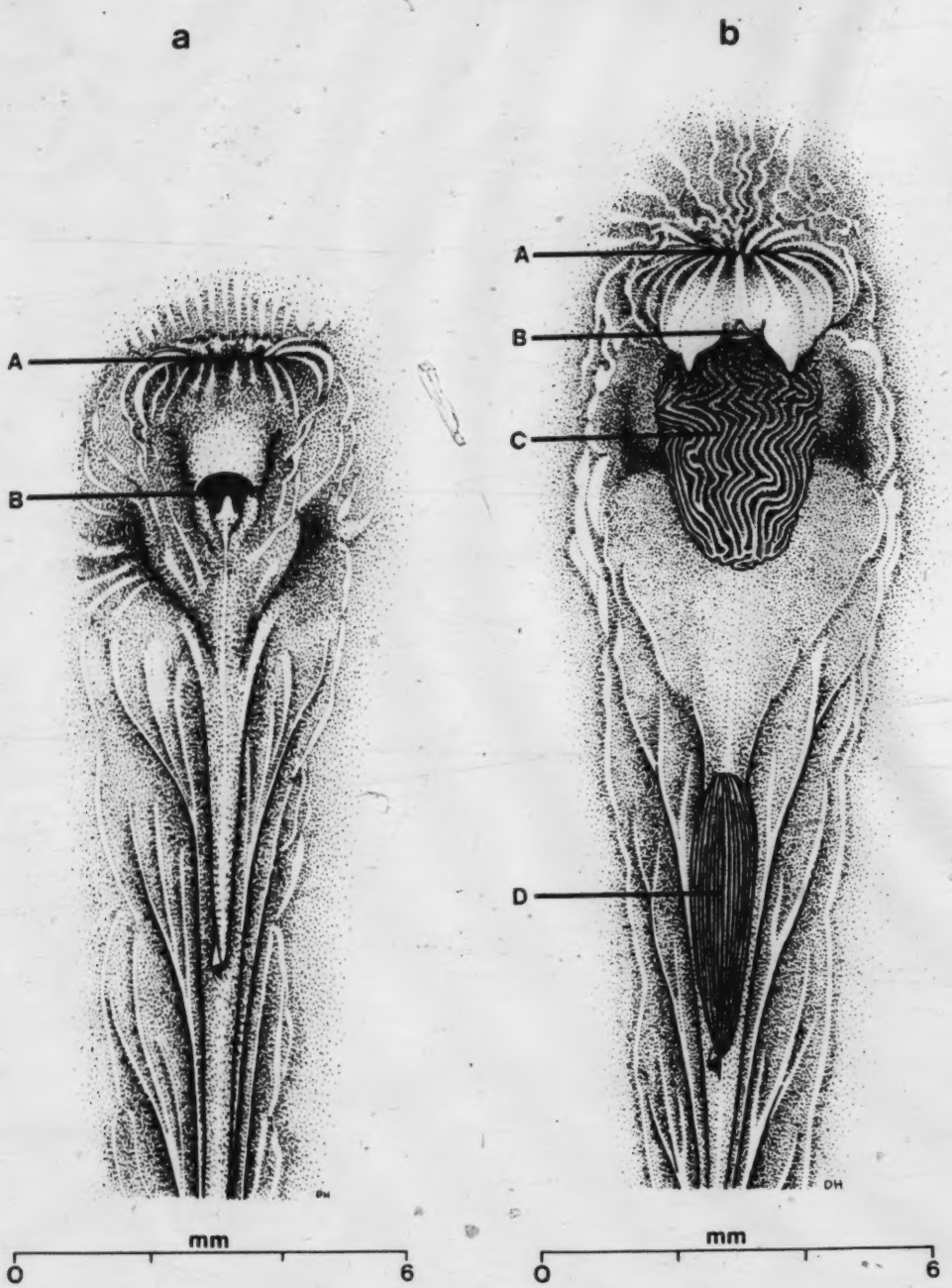
FIGURE 3.

Ventral views of a female (a) and a male (b) red-lip blenny, illustrating sexual dimorphism in the urogenital region. A, Anus; B, Urogenital orifice; C, AFF 1; D, AFF 2. Drawing by Diane Hollingdale.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D
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6

C. Histology and Histochemistry:

AFF and gonad samples from male specimens collected at all lunar phases were fixed in Bouin's solution. After 2 weeks in the fixative, the tissues were washed and stored in 70 % ethanol. In Edmonton, tissue samples were dehyrated, cleared, infiltrated and embedded in Historesin (LKB Bromma).

Two μm tranverse sections were made on a Sorvall JB-4A microtome using glass knives. For general histology, the sections were stained with Harris hematoxylin and eosin. The histological preparations were examined at 100x and 400x magnification using a Wild Leitz compound microscope. Photomicrographs were made using a Wild MPS51 camera fitted to the microscope. Various color correction filters (placed over the light source) were used to obtain accurate color prints.

Three histochemical procedures, essentially testing for carbohydrates and proteins, were applied on Bouins-fixed samples to illustrate the different cellular components of the AFF. Two μm plastic sections (prepared as described earlier) from 10 specimens were used for all the tests. The staining procedures were: periodic acid-Schiff, aldehyde-fuchsin with orange G, mercuric bromphenol blue.

Periodic acid-Schiff:

This was applied to demonstrate polysaccharides which stain red. I followed the routine procedure modified for plastic sections described in "Instruction Manual: Staining Procedures For Plastic Embedded Tissue" published by Du Pont

Instruments (1979).

Modified aldehyde-fuchsin with orange G:

This test was used to demonstrate sulphated glycoproteins and to highlight differences between the cell-types within the AFF epithelium. The procedure employed was a composite method from Halami (1952) and the Gomori (1950) method modified by Scott (1952). The modified composite method was as follows :

1. Rinse sections in tap water
2. Treat in acidified 1% $KMnO_4$ for 2 minutes
3. Wash in distilled water
4. Treat in 2 % sodium metabisulphite for 2 minutes
5. Rinse in distilled water
6. Wash in tap water for 2 minutes
7. Stain in aldehyde-fuchsin for 1 hour
8. Wash in 95 % alcohol
9. Rinse in distilled water
10. Stain in Harris' hematoxylin for 1/2 hour
11. Rinse in distilled water
12. Wash in running tap water for 20 minutes
13. Rinse in distilled water
14. Counterstain in orange G for 15 minutes
15. Dehydrate in absolute alcohol for 10 minutes
16. Repeat step 15
17. Clear in Histoclear (National Diagnostics) for 10 minutes
18. Repeat step 17
19. Mount in DePeX (Gurr)

Mercuric bromphenol blue :

This was used to demonstrate proteins which stain blue. I followed the procedure described by Chapman (1975), with the only modification being that the plastic sections used here were left in the stains for longer periods to improve the efficiency of staining.

D. Histometry

PAS-stained AFF sections were used in a histometric investigation to determine changes in size and number of epithelial cellular components over the lunar cycle falling within August 1987. This particular cycle from the sampling study was randomly chosen. Ten specimens, chosen randomly from each lunar phase, were used.

Only transverse sections taken through the centre of the AFF were measured (400x magnification). Various parameters were determined using an ocular micrometer and ocular grid. Five separate measurements were conducted for each parameter on one section for each fish and a mean calculated. This mean value per fish was then used to calculate mean values for the parameter at each lunar phase.

(i) Epidermal tissue height:

Epidermal tissue height was measured from the basement membrane to the tip of the cuticle.

(ii) Epithelial cell height:

The thickness of the layer of epithelial cells found at the base of the epidermis was measured from the basement membrane to the outer surface of the layer.

(iii) Dermal tissue thickness:

The thickness of the dermal layer was measured from the edge of the anal fin spine to the basement membrane of the fold of epidermal tissue located to the immediate right of the spine.

(iv) Epidermal cell density:

The number of epidermal cells falling within 75 μm was determined.

(v) Epidermal cell dimensions:

Length and width of a single epidermal cell from each of five areas was measured and mean cell height and width calculated for each sample.

(vi) Testicular gland cell dimensions:

Ten testes from each lunar phase in the August, 1987 lunar cycle were used. Transverse sections, taken through the middle portion of the testes, were examined at 400x magnification. The diameter of a single gland cell from each of 5 areas was measured on one section for each testis and a mean calculated.

To determine if there was a temporal relationship between the AFF and the testicular gland (TG), a testicular gland index (TGI) was calculated. The same testes used for measuring testicular gland cells were used. Transverse sections, taken through the middle portion of the testes, were examined at 100x and 400x magnification. An ocular square grid was used to measure the areas of the dorsally situated TG and seminiferous tissue (Fig. 4). From serial sections of 5 testes, it is known that as the TG runs the length of the dorsal portion of the testis, it maintains a fairly constant proportional relationship with the seminiferous portion (Table 1). Hence, the fraction of the

whole gonad made up by the TG could be estimated as TGF = cross-sectional area of TG / total cross-sectional area of testis. From this the TGI was calculated as TGI= TGF x GSI. A seminiferous tissue index (SI) was determined using similar calculations.

FIGURE 4.

Cross-sectional schematic of testis showing division into glandular and seminiferous components. A, seminiferous tissue; B, testicular gland; C, artery; D, seminiferous duct; E, vein; d, dorsal aspect; v, ventral aspect.

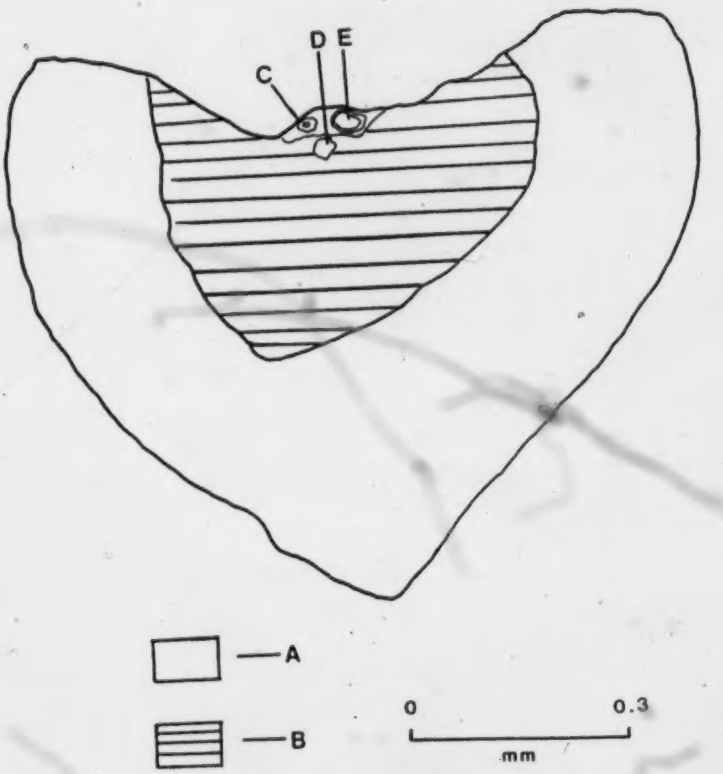

D
E
C
d
v
A
B
0
0.3
mm

TABLE 1

Ratio Of Gland Size To Seminiferous Tissue Size Along Testis

Specimen no.	Anterior	Middle	Posterior	Mean +/ SE
1	0.276	0.267	0.284	0.276 +/- 0.005
2	0.196	0.192	0.200	0.196 +/- 0.002
3	0.294	0.282	0.305	0.294 +/- 0.007
4	0.399	0.407	0.414	0.407 +/- 0.004
5	0.448	0.464	0.456	0.456 +/- 0.005

E. Electron Microscopy:

An ultrastructural study was conducted on the AFF epithelium of a sexually mature male (SL= 72.0 mm; 6.6 g, collected at new moon) to characterize major cellular components.

The AFF was cut into small pieces (about 1 mm thickness) and placed into phosphate buffered glutaraldehyde for immediate fixation. This fixative, recommended for a wide variety of marine organisms (Cloney and Florey, 1968), consisted of 2.5 % glutaraldehyde, 0.2 M Millonig's phosphate buffer and 0.14 M NaCl. The tissue pieces were left in this primary fixative for 1 hour in an ice-bath, after which they were rinsed in a cold buffer solution (0.2 M Millonig's phosphate buffer and 0.3 M NaCl) for 15 minutes. The pieces were then left in 2% osmium tetroxide in 0.2 M Millonig's phosphate buffer for secondary fixation for 1.5 hours, again in an ice-bath.

After several rinses in the cold buffer solution, the samples were dehydrated in ethanol, cleared and infiltrated with propylene oxide and embedded in Araldite 502 (Ladd).

Thick and thin sections were made on a Sorvall MT2-B ultra microtome, using glass and diamond knives respectively. These sections were stained with saturated uranyl acetate in 50 % ethanol followed by lead citrate (Reynolds, 1963) for 10 minutes. Material was examined using a Phillips EM 210 electron microscope.

F. Effect of Androgens on AFF Morphology:

Thirty sexually mature males of comparable size were collected over a 2-day period at the last quarter (LQ) phase of the lunar cycle in October, 1987 and divided randomly into 3 groups which were maintained outdoors in identical, sheltered, 5400-litre concrete tanks under ambient light. An assortment of coral rocks provided habitats for the fishes. Water was flowing (5 litres/minute) at a constant temperature of 28°C. Fish fed on algal growth within the tanks, scraping it off the floor, walls and coral rocks. One to two days after being placed in the tanks the animals set up territories which they apparently maintained up to the end of the experimental period. A period of one week elapsed between the time of capture and the onset of treatments.

Control males were injected intraperitoneally with 50 µl saline (0.9 % NaCl; two drops Tween 80/100 ml), whereas test males received 10 µg/g Testosterone (Sigma) or 11-ketotestosterone (Sigma) suspended in the saline. Fish were anaesthetized (2-phenoxyethanol at 0.01 % in sea-water) prior to injection and revived in aerated sea-water before being returned to the stock tank.

Each fish received four treatments, once every four days. Three days after the last treatment, the fishes were sacrificed and morphometric measurements made. The AFFs were fixed in Bouin's solution for subsequent histological and

histochemical comparisons. Histometrical measurements, similar to that described in the previous section, were also completed.

G. Statistical Analyses:

Data from the histometric study and androgen treatment experiments reported here were analysed by one-factor analysis of variance (ANOVA) and Tukey's multiple-comparison test (Zar, 1984).

The significance of correlation coefficients between testicular indices and AWI data was determined using a two-tailed Student's t-test (Zar,1984).

IV. RESULTS

A. Gross Morphology of the Urogenital Region:

The AFF (Fig. 2) in O. atlanticus (Fig. 3) is found only in males. The urogenital orifice opens at the tip of a papilla in males while in females it is partly occluded by a small hood of tissue.

With the exception of rare variants, all blenniids possess two anal fin spines (Porter, 1979). The number of segmented anal fin rays in blenniids, however, may vary from 10 to 119 (Porter, 1979). Each male redlip blenny has two AFFs which are present even in sexually immature specimens. The first and larger AFF is associated with the first anal spine while the smaller AFF encloses the second spine.

The surfaces of both AFFs are thrown into invaginations which are more apparent in sexually mature males. Smaller males often exhibit rugosity in only the first AFF. Normally, the color of the AFFs closely matches that of the body, i.e. a dark chocolate brown. However, swollen AFFs often take on a distinct pinkish hue. It has been noted (Nursall, personal communication) that a similar pinkish hue suffuses the head of a blenny during reproductive behavior.

The fish can move its anal fin spines and hence the AFFs by means of fin ray musculature around the base of the spines. This movement seems to be limited to the sagittal plane and was only observed when the fishes were handled.

B. LUNAR SPAWNING CYCLE :

GSI of females was greatest during FQ and FM (Fig. 5). However, the difference in mean GSIs between FQ and FM was probably more pronounced than Fig. 5 suggests. The largest variation in GSIs over the sampling period was observed at each FM phase. This variation was positively skewed, with the mode occurring between a GSI of 0.948 and 1.656. Despite this, more than 40% of all females sampled at FM had GSIs greater than 2.00. Hence, even though some very low GSIs were observed, several very large values were also recorded, suggesting that spawning was occurring during this period. The largest individual GSIs were recorded at FQ, indicating that GSIs peaked at this period and were probably already beginning to drop by FM. In two of the three months for which data are available, there was a large decrease in GSIs in the week between FM and LQ. The pattern for July was similar though less dramatic. These large drops further suggest that spawning continued through FM and ended sometime before LQ. These data suggest that peak spawning activity was centred around the FM.

Lunar GSI changes in males were not as consistent as those of females (Fig. 6a). However, for each lunar cycle, the lowest mean GSI value occurred at FQ. If all the GSI values at each stage of the lunar cycle are taken together, this decrease in GSI at FQ is evident (Fig. 6b). The complicated male GSI profile may have been due to asynchronous changes in the glandular and seminiferous

FIGURE 5.

GSI changes (±SEM) of females from June-September, 1987. No data are available for the FQ period occurring in July. NM new moon; FQ first quarter; FM full moon; LQ last quarter. N = 15 for each lunar phase.

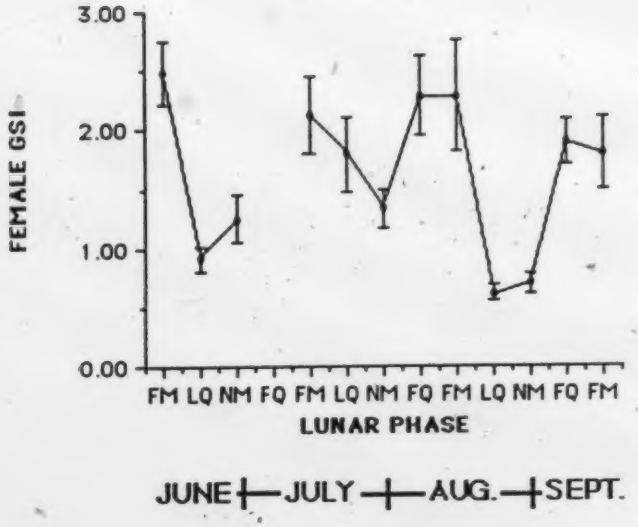
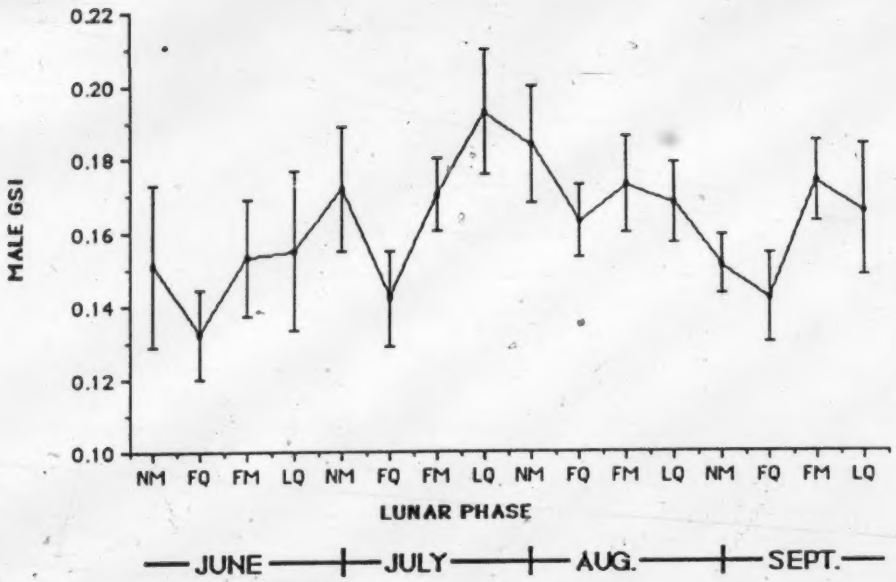

3.00
2.00
1.00
0.00
FEMALE GSI
FM LQ NM FQ FM LQ NM FQ FM LQ NM FQ FM
LUNAR PHASE
JUNE
JULY
AUG.
SEPT.

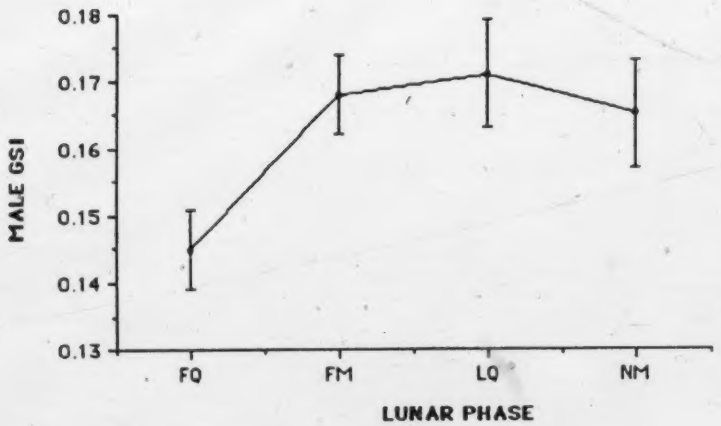
FIGURE 6.

GSI of male (±SEM) over the sampling period. (a) Profile of male GSI over the 4-month sampling period. N = 15 for each lunar phase. (b) GSI data from the 4 lunar cycles are pooled to produce a profile for a single, composite lunar cycle.

a


0.22
0.20
0.18
0.16
0.14
0.12
0.10
MALE GSI
NM FQ FM LQ NM FQ FM LQ NM FQ FM LQ NM FQ FM LQ
LUNAR PHASE
JUNE
JULY
AUG.
SEPT.

b


0.18
0.17
0.16
0.15
0.14
0.13
MALE GSI
FQ FM LQ NM
LUNAR PHASE

components of the testis.

As indicated by AWI, AFF size shows a lunar periodicity, the lowest weights occurring at NM and the largest at FM (Fig. 7). There appears to be a gradual increase in FM peaks and gradual decrease in NM values from the first lunar cycle to the fourth. This pattern is similar to the lunar changes in AAI (Fig. 8).

FIGURE 7.

Anal-fin-fold weight index (AWI; ±SEM) over the sampling period. N = 15 for each lunar phase.

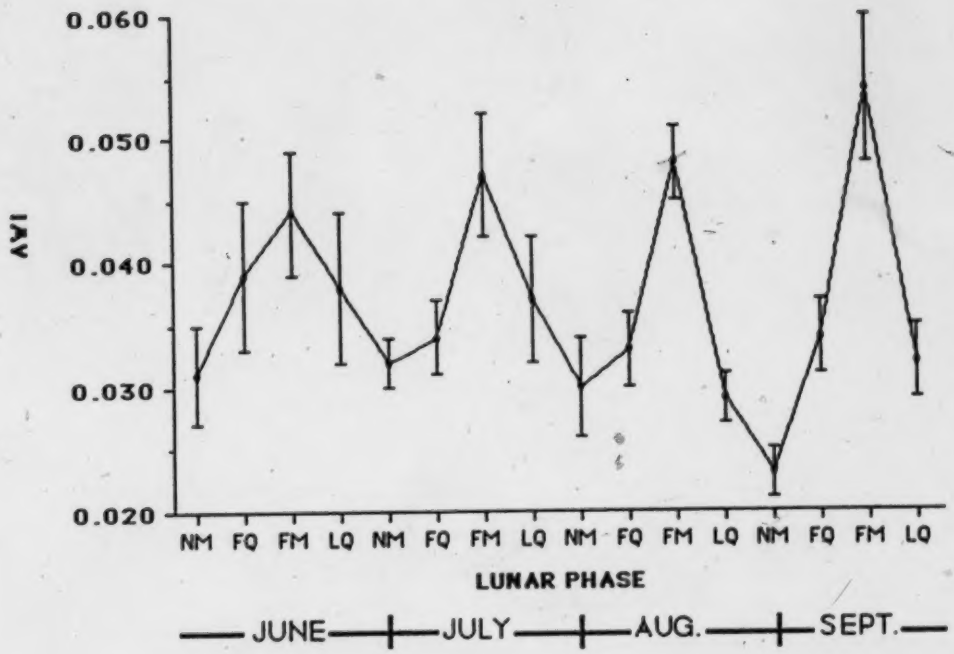
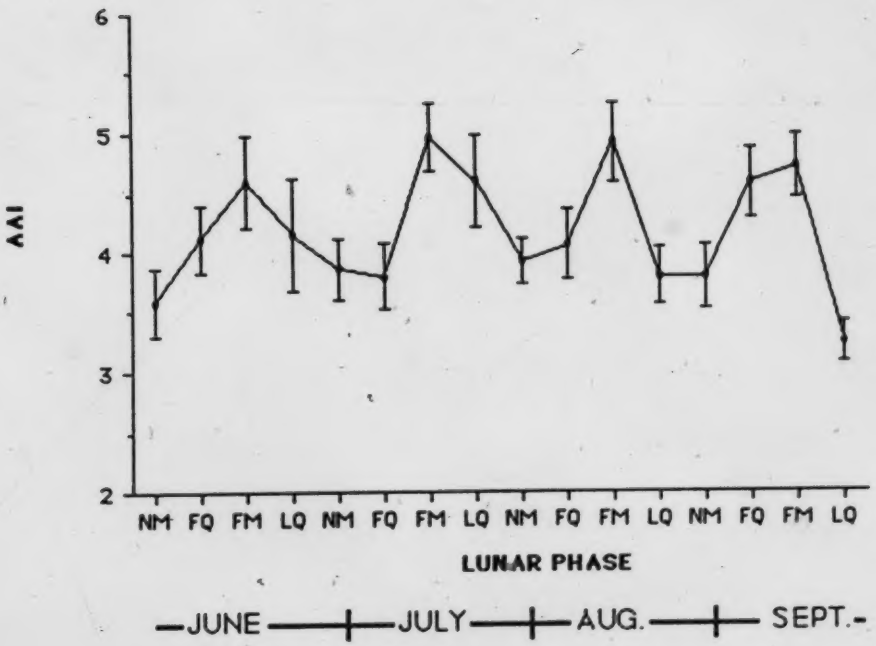

0.060
0.050
0.040
0.030
0.020
IAV
NM FQ FM LQ NM FQ FM LQ NM FQ FM LQ NM FQ FM LQ
LUNAR PHASE
JUNE
JULY
AUG.
SEPT.

FIGURE 8.

Anal-fin-fold area index (AAI; ±SEM) over the sampling period. N = 15 for each lunar phase.


6
5
4
3
2
AAI
NM FQ FM LQ NM FQ FM LQ NM FQ FM LQ NM FQ FM LQ
LUNAR PHASE
JUNE
JULY
AUG.
SEPT.

C. Histology:

AFF:

Both anterior and posterior AFFs consist of an epidermal layer covering loose connective tissue which completely encloses the anal fin spines (Fig. 9). As for vertebrates in general (Bullock and Roberts, 1974), this connective tissue is composed mainly of collagen fibres which form a matrix containing a rich supply of blood vessels and some chromatophores. The connective tissue is contiguous with a basement membrane (BM) which separates it from the epidermis.

As is typical of teleost skin, a supra-epithelial layer, the cuticle, covers the epidermis (Fig. 10). The cuticle appears to be 1 μm thick and has been proposed to be a complex of sloughed cells, mucus (originating both from mucous and other epidermal cells) and cell protoplasm (Bullock and Roberts, 1974).

In this thesis, the nomenclature for epithelial cell types devised by Whitear (1971) and Mittal et al. (1979) is adopted. The epidermal tissue consists of three cell types: basic epithelial cells and two secretory cell types, serous and mucous cells. The epidermis is stratified with basic epithelial cells forming the basal layer adjacent to the BM. Basic epithelial cells are the main structural component of the epidermis. Such cells are typically polygonal, 5 μm in diameter and occasionally occupy layers above the basal layer. Their nuclei are generally very large (up to 3 μm diameter), and occupy much of the cell.

FIGURE 9.

(a) Schematic of frontal section of unswollen AFF showing the first anal-fin-spine (C) surrounded by dermal or connective tissue (B) with an overlying epidermal layer (A).(b) Schematic of cross section of an unswollen AFF.

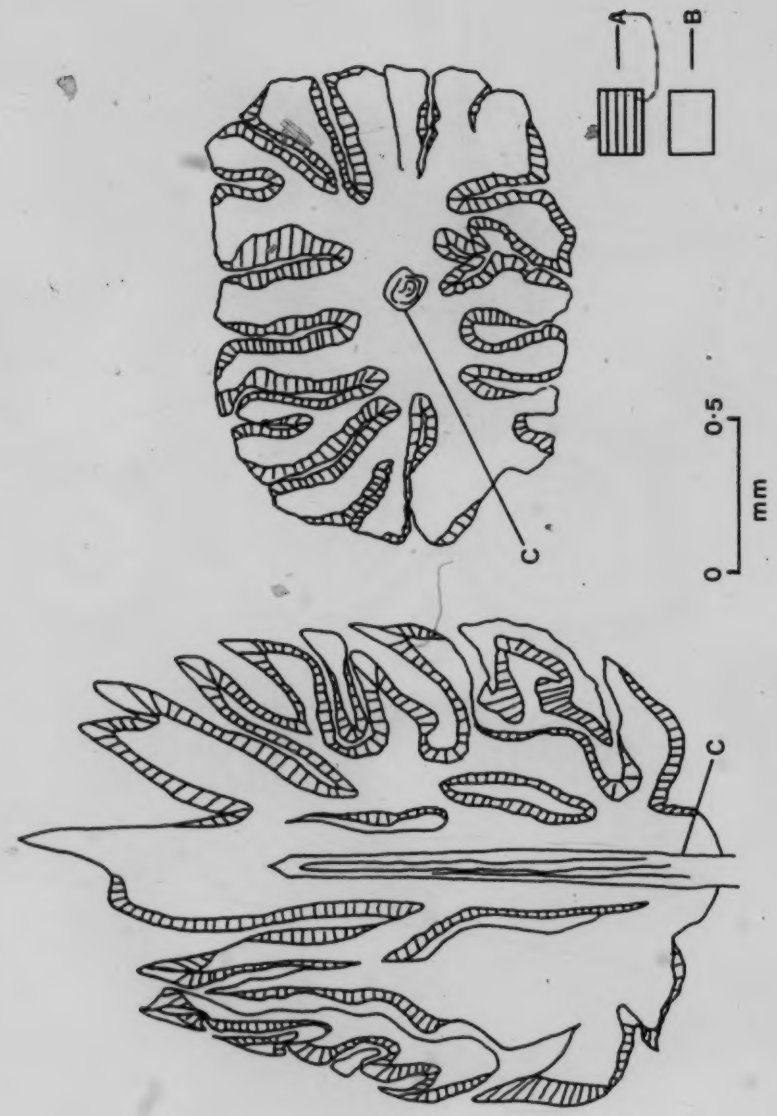
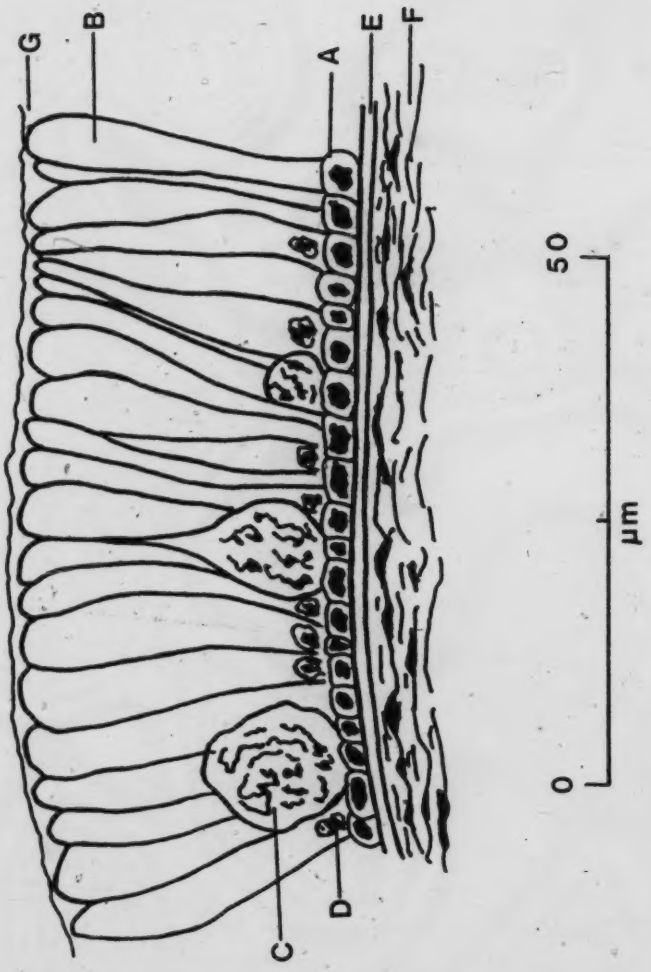

A
B
b
C
0·5
0
mm
a
C

FIGURE 10.

Line drawing of AFF epidermal tissue, typically found in a swollen AFF. A, basic epithelial cell; B, serous cell; C, mucous goblet cell; D, nucleus of serous cell; E, basement membrane; F, dermal layer/connective tissue; G, cuticle.


G
B
A
E
F
C
D
0
50
µm

The more prominent of the two secretory cells are the "serous" cells. They rise from the basal layer to the surface where they appear to be contiguous with the cuticle. Serous cells are elongate, spindle-shaped, and have their long axes perpendicular to the BM. In an unswollen AFF, they average 26 μm in length and 5 μm in width. The nucleus is located basally and the cytoplasm is granular. These granules have a strong affinity for eosin, an acid dye, thereby suggesting a proteinaceous nature. It was not apparent, at the light microscopy level, whether these unicellular glands open to the surface via pores, etc. Serous cells are probably merocrine (i.e. the product is secreted by exocytosis without visible damage to the secretory cell). There is no evidence for ducts into which the glands empty.

The second unicellular gland type, the mucous cells, are the least common of the epidermal cells and are usually found in the lower to middle layers. They are generally ovoid and from 5 to 20 μm in diameter. In large mucous cells, the nucleus is displaced basally with large globules of secretory material occupying most of the cytoplasm. Occasionally, larger flask-shaped mucous cells (to 20 μm diameter) were observed with apical "necks" leading to the surface. The cytomorphological features of these cells are similar to those described for mucous goblet cells (Whitear, 1986) which generally display an apocrine (i.e the product is released by sloughing off the apical portion of the cell) form of secretion (Whitear, 1986).

Changes in AFF size associated with the lunar cycle appear to be largely due to changes in epidermal tissue (Plate 1a and 1b). During the NM phase (Fig. 11a; Plate 2a), at which no spawning activity was observed, basic epithelial cells are most abundant and extend 2-3 layers deep from the BM. The serous cells at this stage are tall and narrow and have a granular composition; they could not be found in several specimens. Mucous cells are entirely absent at this phase of the lunar cycle.

By FQ (Fig. 11b; Plate 2b), the basic epithelial cells remain fairly abundant but now are limited almost completely to a single basal layer. Serous cells are taller and wider than at NM. The granules are not easily identifiable at this stage. This could be attributable to an aggregation of granules in many cells. Some mucous cells are present although these are very small (5 μm diameter) and interspersed among the epithelial cells.

In the FM phase, changes first observed at FQ are further developed (Fig. 11c; Plate 2c). The basic epithelial cells are now reduced in numbers and restricted to a layer along the BM. Serous cells are uniformly large and are clearly the most prominent of the three cell types. Their cellular contents appear to consist of a single, homogeneous mass which could simply be due to a very intense aggregation of granules. Mucous cells now are abundant, generally larger than at FQ (15 to 20 μm diameter) and dispersed both in the lower and middle layers.

PLATE 1.

(a) Cross-sectional view of an unswollen AFF stained with Harris hematoxylin and eosin (HE; SL = 70.5 mm). (b) Cross-sectional view of a swollen AFF (HE; SL = 72.2 mm). Both plates 100x; CC10C and CC20C filters. Scale represents 0.2 mm.

a

b

FIGURE 11.

Composite line drawings illustrating changes in AFF epidermis over one lunar cycle. (a) Epidermis at new moon. (b) Epidermis at first quarter. (c) Epidermis at full moon. (d) Epidermis at last quarter.

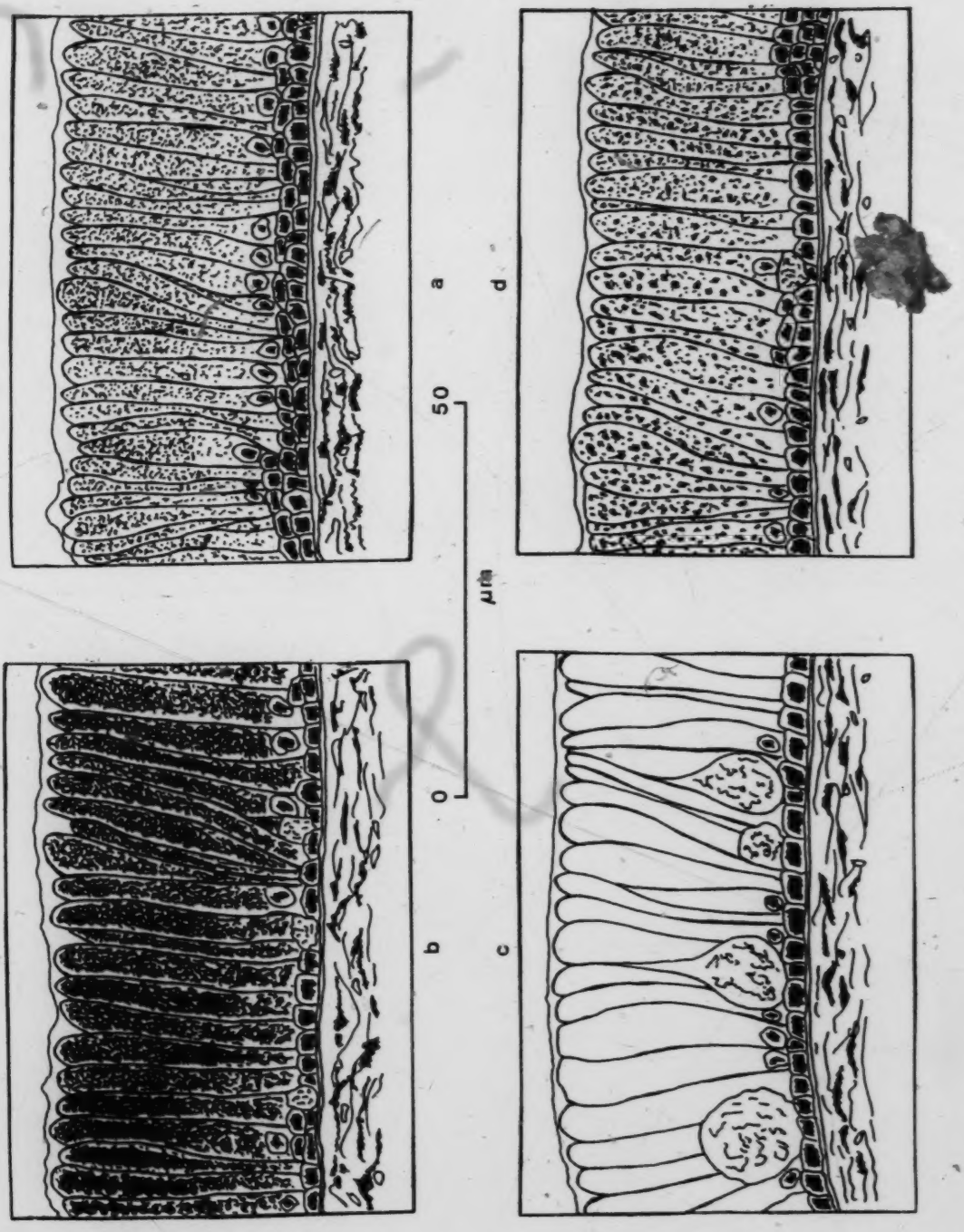
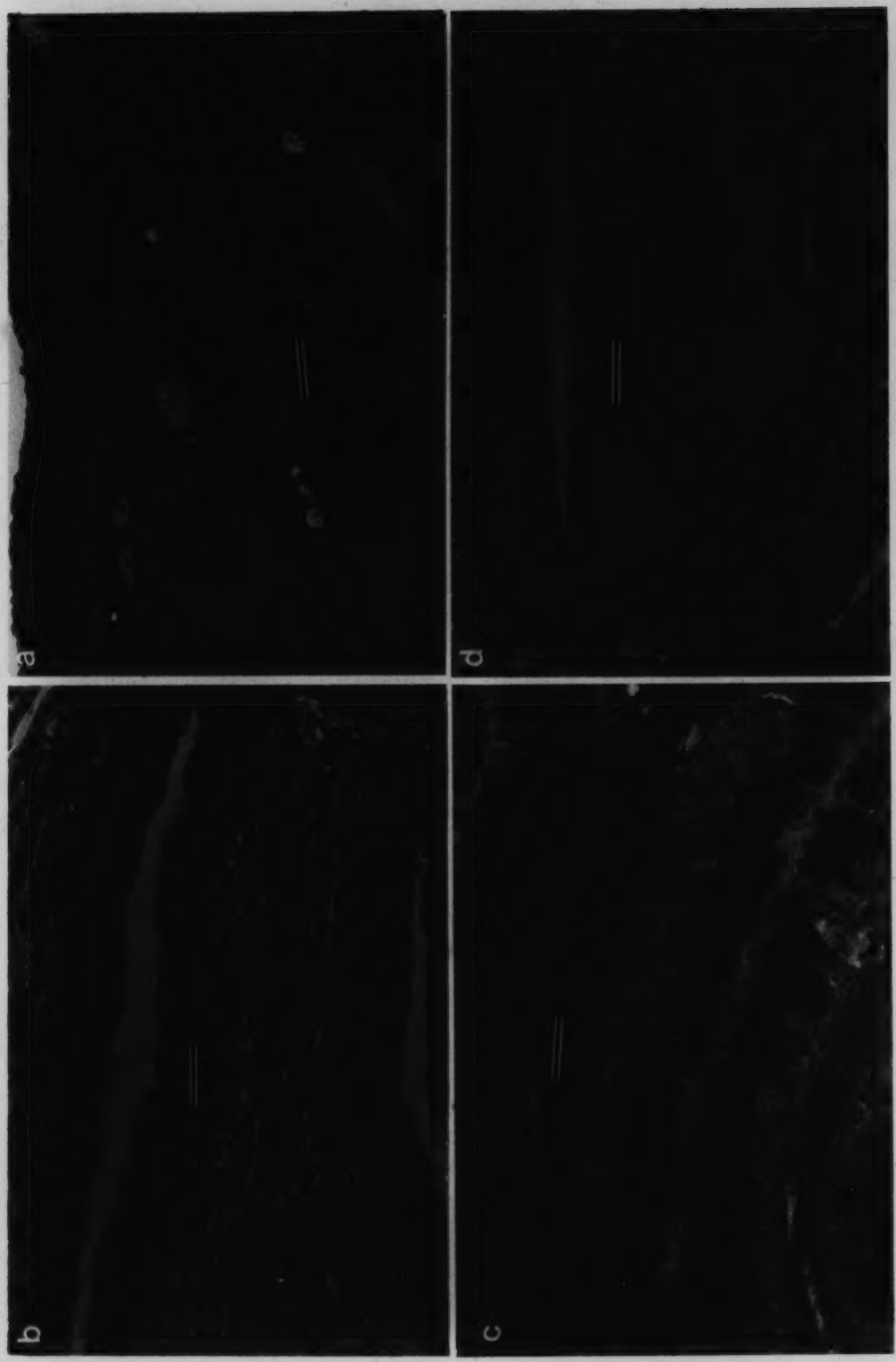

a
d
50
μm
0
b
c

PLATE 2.

Photomicrographs of AFF epidermal changes over the August, 1987 lunar cycle. All plates 400x. (a) Epidermis at new moon (SL = 77.0 mm; HE; CC10C & CC20C filters). (b) Epidermis at first quarter (SL = 69.5 mm; HE; CC10C & CC20C filters). (c) Epidermis at full moon. Tissue stained following PAS procedure with Harris hematoxylin counterstain (PAS & HE; SL = 70.0 mm; 80A filter). (d) Epidermis at last quarter (SL = 73.4 mm; HE; CC10C & CC20C filters). Scale represents 20 μm.


a
d
b
c

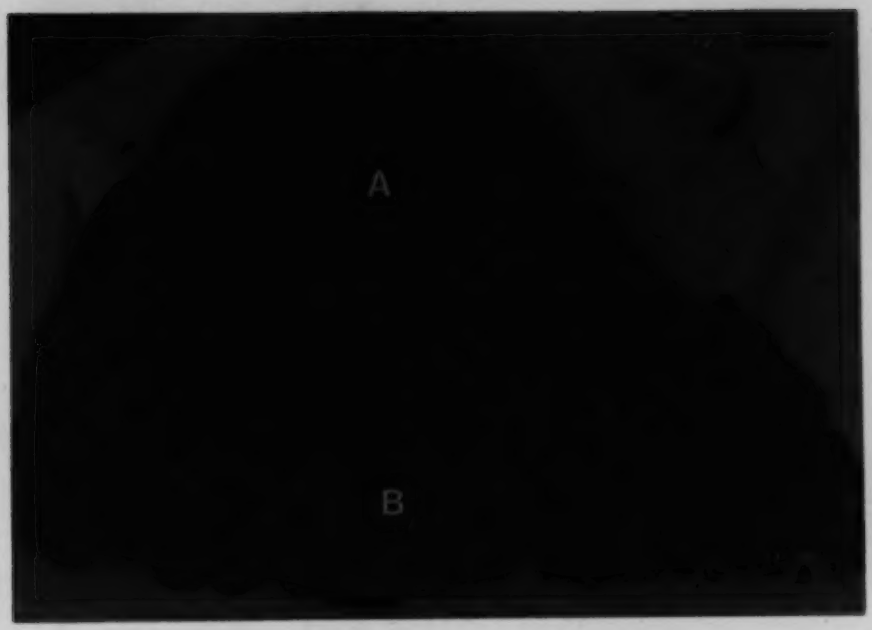
With the transition from FM to LQ, the cytological composition of the AFF epidermis changes considerably. The abundance of basic epithelial cells is highly variable, some AFFs having the low numbers typical of FM and others having more. Serous cells generally decrease in size although quite a few large cells (to 28 μm height) are still present. Serous cell contents, however, are very obviously granular at this point. The few mucous cells present are very small (5 to 7 μm diameter).

Testis:

As I reported earlier, the testis of O. atlanticus consists of glandular and seminiferous components (Plate 3 and Fig. 4). A large artery and vein lie close to the efferent duct (Fig. 4). The dorsally situated gland is apparently composed mainly of a single type of polyhedral glandular cell (17 to 25 μm diameter) with a large nucleus and finely granular cytoplasm. These cells appear to be aggregated into clusters with blood capillaries in the interstices (as in most endocrine organs) but this is not always evident within or between specimens. Within many clusters, there is a ductule through which sperm travel. Microvilli-like processes from the glandular cells were sometimes seen projecting into the lumen of the ductule. Not

PLATE 3.

Photomicrograph of a cross-section of testis sampled at first quarter, showing division into glandular (A) and seminiferous (B) components. Tissue section stained with HE. Plate taken at 100x magnification using a CC20C filter. Scale represents 0.1 mm.

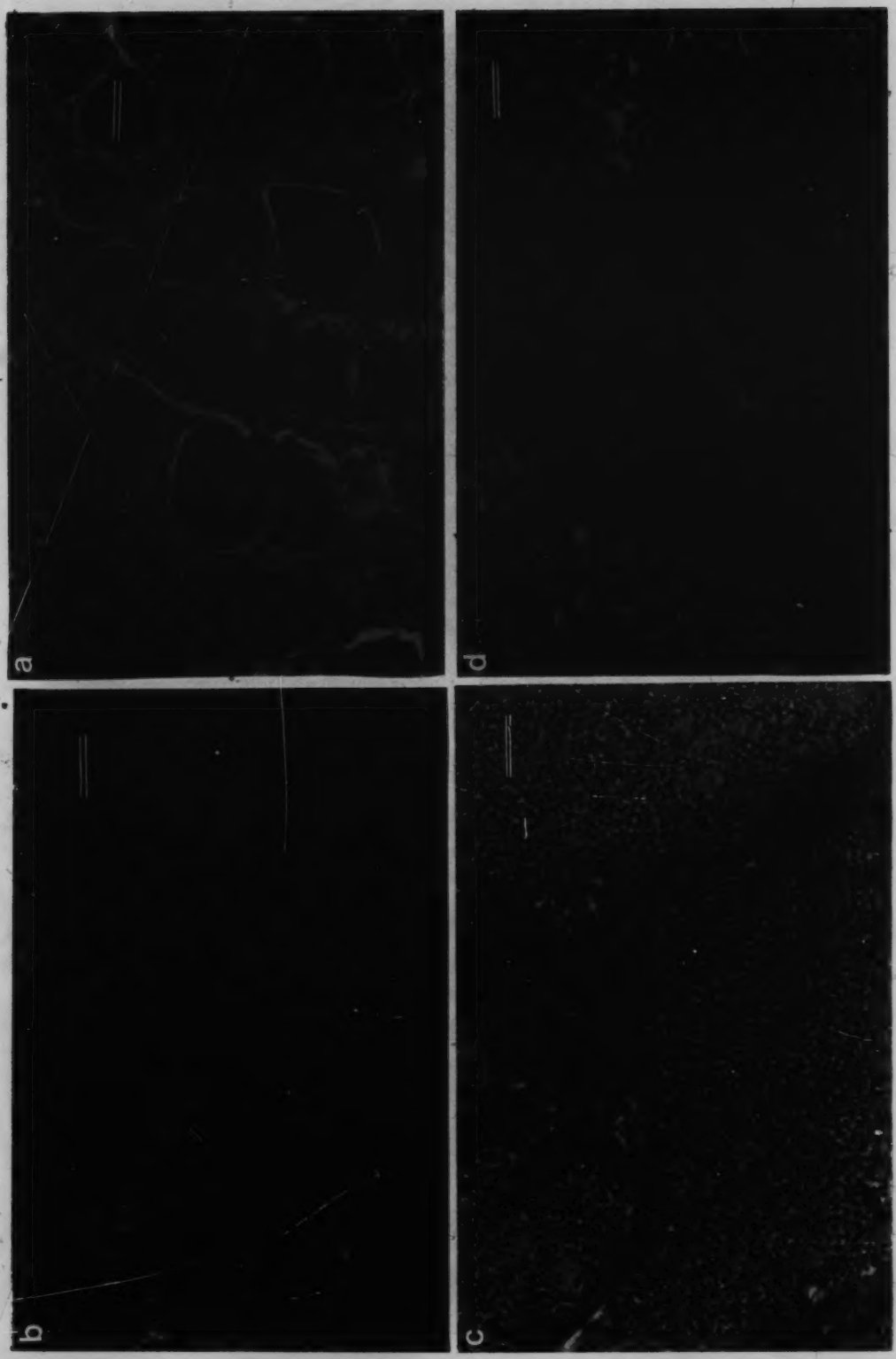

A
B

all the glandular cells feed into ductules. Quite a number were observed tightly packed together with no apparent organization. All the glandular cells are very closely associated with blood capillaries.

The seminiferous component is of a tubular nature with each tubule containing a number of cysts which in turn contain cells at similar stages of development (Plate 3). Hence, within cysts, all the germinal elements are at the same developmental stage but the stage of this development varies from cyst to cyst. Within a testis, all stages of spermatogenesis can be observed. Nonetheless, the proportions of the various germinal stages vary according to the lunar cycle. At NM (Plate 4a), most of the cysts contain spermatocytes or spermatids with only small numbers of cysts having spermatozoa or spermatogonia. At FQ (Plate 4b), a large proportion of the cysts contain either spermatids or spermatozoa. Therefore, the process of spermiogenesis is very prevalent at this lunar phase. By FM (Plate 4c), the lumen of many of the tubules are filled with sperm and surrounded by cysts containing spermatids, spermatocytes and spermatogonia. At LQ (Plate 4d) there is a predominance of cysts with spermatocytes or spermatids. Very few cysts, all of which are small, contain spermatozoa. These observations indicate that maximal sperm numbers occur at FM, which reinforces the link between spawning activity and the lunar cycle.

PLATE 4.

Photomicrographs of seminiferous tissue, illustrating changes over the August, 1987 lunar cycle. All plates 400x; HE; CC10C and CC20C filters. (a) Seminiferous tissue at new moon (SL = 71.7 mm). (b) Seminiferous tissue at first quarter (SL = 80.3 mm). (c) Seminiferous tissue at full moon (SL = 73.2 mm). (d) Seminiferous tissue at last quarter (SL = 75.3 mm). Scale represents 20 μm.


a
d
b
c

D. Histometry:

As reported in the previous section, major changes occur in the AFF epidermal layer over each lunar cycle. The epidermal layer is significantly ($p<0.05$) thicker at FM than at the other phases (Fig. 12a). Associated with this is a change in the thickness of the basic epithelial cell layer over the lunar cycle, with NM and LQ layers being significantly thicker than those at FQ and FM (Fig. 12b).

At FM, the number of serous cells counted within a 75 μm strip of tissue was significantly lower than at FQ and NM (Fig. 13a), whereas maximum serous cell height (Fig. 13b) and width (Fig. 13c) was observed at FM.

Mucous cells were not found at NM, increased in number at FQ and FM, and decreased significantly at LQ (Fig. 14).

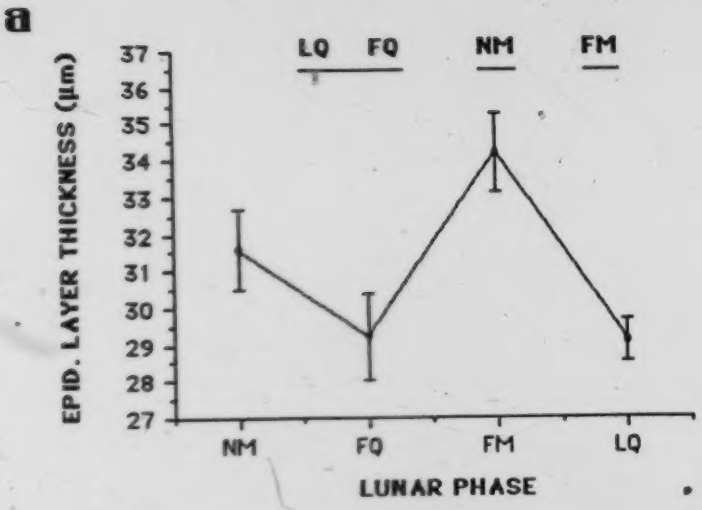
The dermal tissue layer was significantly thicker at FQ than at the other phases (Fig. 15).

Male GSI changes during the August lunar cycle showed no obvious pattern (Fig. 16a). The same was true for both the seminiferous tissue index (SI; Fig. 16b) and the testicular gland index (TGI; Fig. 16c). Variations in the ratio of TG to testis (Fig. 17a) and in gland cell size (Fig. 17b) were readily apparent but not obviously related to the lunar cycle.

The relationship between AFF and testis size was examined at NM and FM. The correlation between AWI and GSI was significant at NM but not at FM (Fig. 18; t-test, $p<0.01$). Changes in the size of the seminiferous component of

FIGURE 12.

Changes in AFF epidermal tissue during the August, 1987 lunar cycle. (a) Thickness of AFF epidermis (μm). (b) Thickness of AFF basic epithelial cell layer (μm). Vertical bars represent standard errors about the mean. Groups not underlined by the same line are significantly different ($p<0.05$). N = 10 for each lunar phase.


a
LQ FQ
NM
FM
EPID. LAYER THICKNESS (µm)
37
36
35
34
33
32
31
30
29
28
27
NM
FQ
FM
LQ
LUNAR PHASE

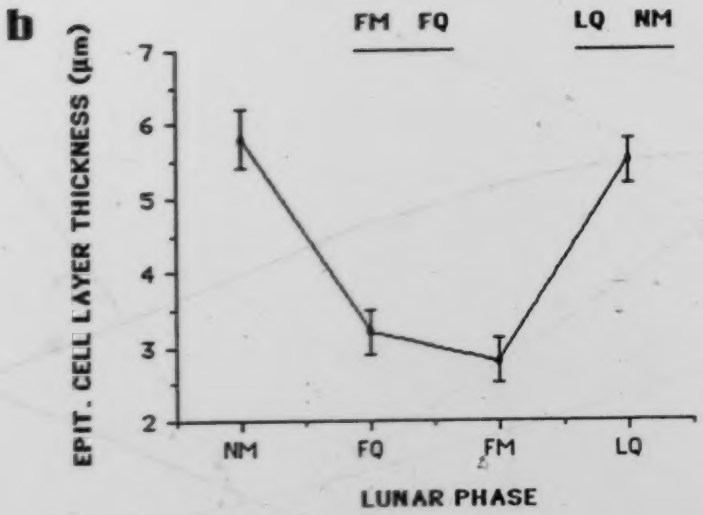

b
FM FQ
LQ NM
EPIT. CELL LAYER THICKNESS (µm)
7
6
5
4
3
2
NM
FQ
FM
LQ
LUNAR PHASE

FIGURE 13.

Size and condition of AFF serous cells during the August, 1987 lunar cycle. (a) Serous cell density (cell count in a 75 μm strip of epidermis). (b) Serous cell height (μm). (c) Serous cell width (μm). Vertical bars represent standard errors about the mean. Groups not underlined by the same line are significantly different ($p<0.05$). N = 10 for each lunar phase.

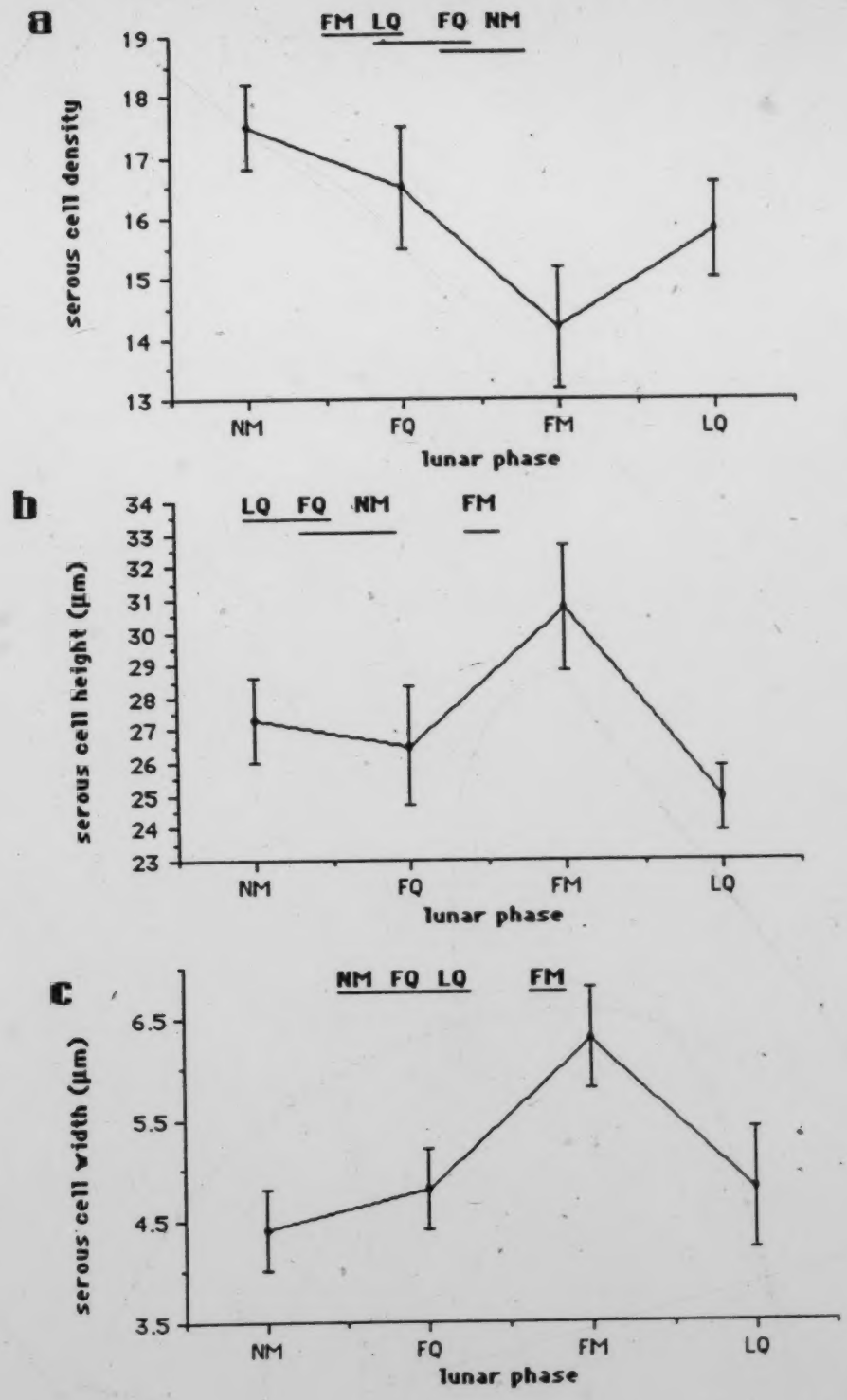

a
FM LQ FQ NM
serous cell density
19
18
17
16
15
14
13
NM
FQ
FM
LQ
lunar phase
b
LQ FQ NM
FM
serous cell height (µm)
34
33
32
31
30
29
28
27
26
25
24
23
NM
FQ
FM
LQ
lunar phase
c
NM FQ LQ
FM
serous cell width (µm)
6.5
5.5
4.5
3.5
NM
FQ
FM
LQ
lunar phase

FIGURE 14.

Mucous cell density (±SEM) during the August, 1987 lunar cycle. The density is represented here as a count of mucous cells within a 75 μm strip of epidermal tissue. Groups not underlined by the same line are significantly different ($p<0.05$). N = 10 for each lunar phase.

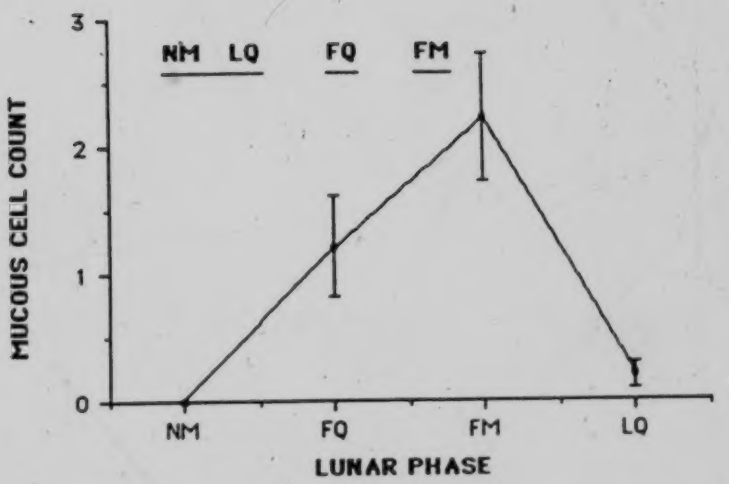

NM LQ
FQ
FM
MUCOUS CELL COUNT
3
2
1
0
NM
FQ
FM
LQ
LUNAR PHASE

FIGURE 15.

AFF dermal tissue thickness (±SEM) during the August, 1987 lunar cycle. Groups not underlined by the same line are significantly different ($p<0.05$). N = 10 for each lunar phase.

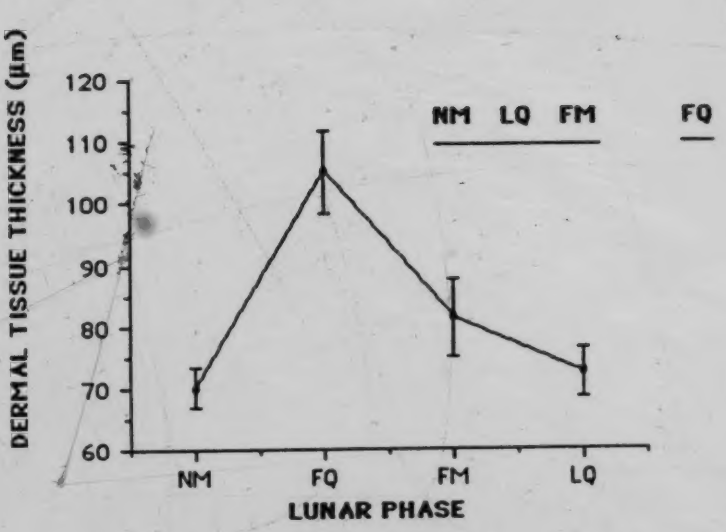
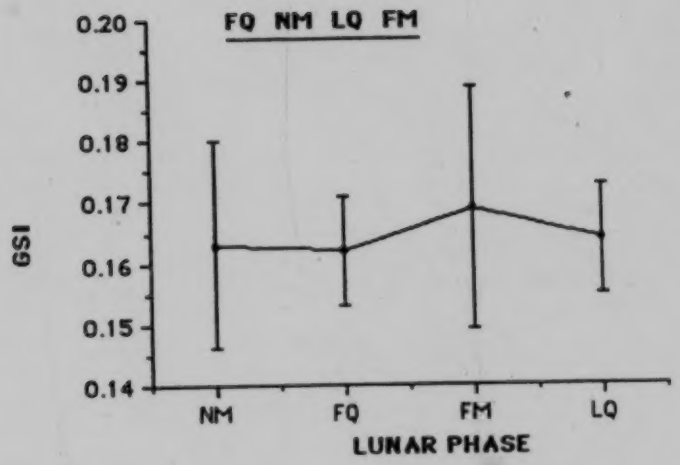

DERMAL TISSUE THICKNESS (µm)
120
110
100
90
80
70
60
NM
FQ
FM
LQ
LUNAR PHASE
NM LQ FM FQ

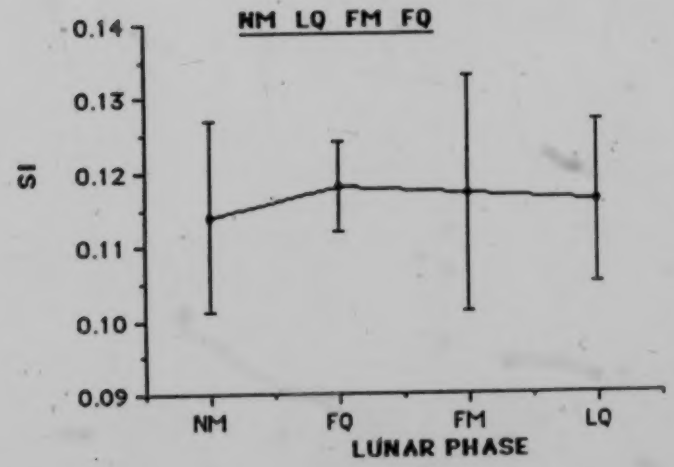
FIGURE 16.

Relative size of testis (and its 2 components) during the August, 1987 sampling period. (a) Male GSI. (b) Seminiferous tissue index (SI). (c) Testicular gland index (TGI). Vertical bars represent standard errors about the mean. Groups underlined by the same line are not significantly different ($p<0.05$). N = 10 for each lunar phase.

a


FQ NM LQ FM
0.20
0.19
0.18
0.17
0.16
0.15
0.14
GSI
NM
FQ
FM
LQ
LUNAR PHASE

b


NM LQ FM FQ
0.14
0.13
0.12
0.11
0.10
0.09
SI
NM
FQ
FM
LQ
LUNAR PHASE

c

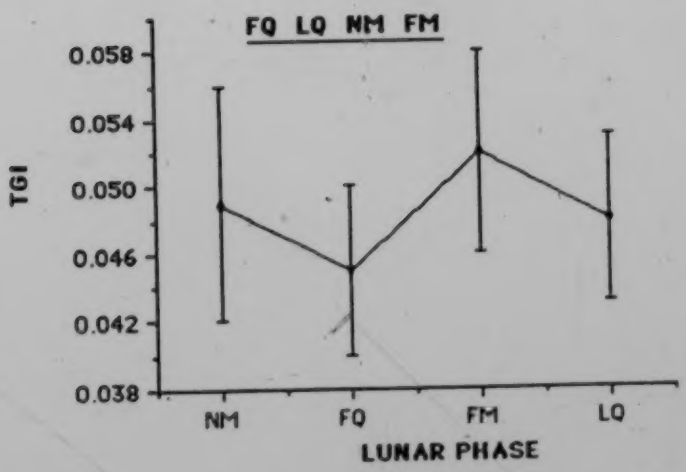
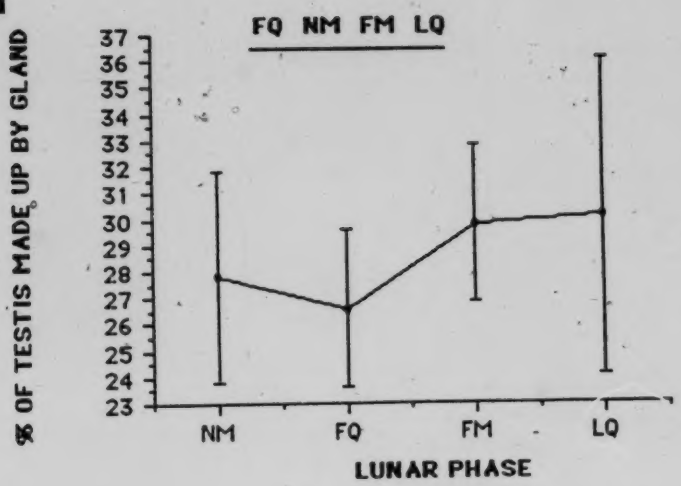

FQ LQ NM FM
0.058
0.054
0.050
0.046
0.042
0.038
TGI
NM
FQ
FM
LQ
LUNAR PHASE

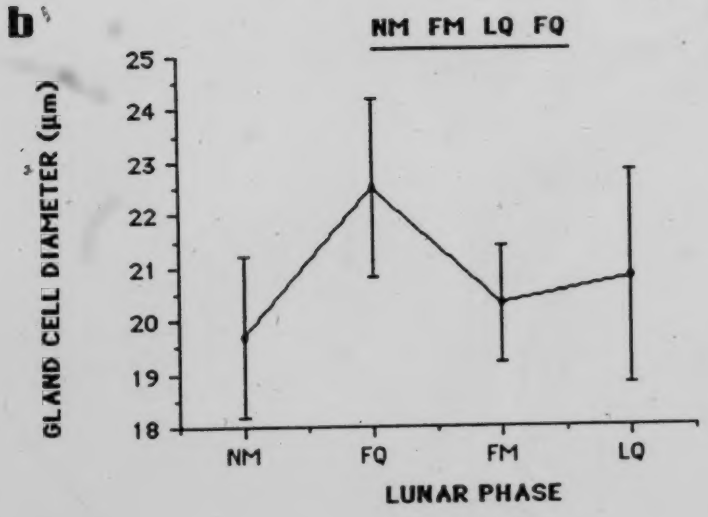
FIGURE 17.

Changes in the testicular gland during the August, 1987 lunar cycle. (a) Percentage of testis which was testicular gland. (b) Changes in testicular gland cell diameter (μm). Vertical bars represent standard errors about the mean. Groups underlined by the same line are not significantly different. N = 10 for each lunar phase.

a


FQ NM FM LQ
% OF TESTIS MADE UP BY GLAND
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
NM
FQ
FM
LQ
LUNAR PHASE

b

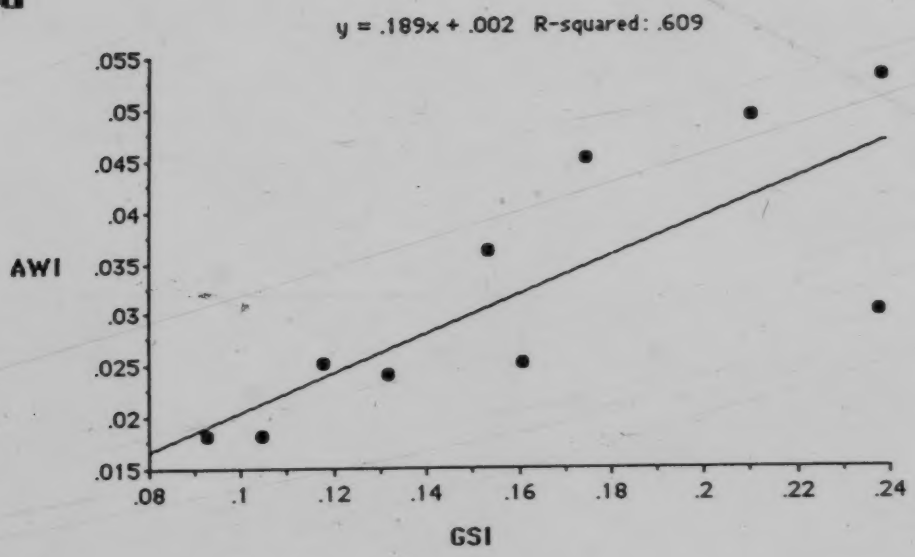

NM FM LQ FQ
GLAND CELL DIAMETER (µm)
25
24
23
22
21
20
19
18
NM
FQ
FM
LQ
LUNAR PHASE

testes were not correlated with changes in AWI (Fig. 19). Changes in AWI were positively correlated with changes in TG size but only at NM (Fig. 20, t-test, $p<0.02$).

FIGURE 18.

Relationship between AWI and GSI in males sampled at NM (a) and FM (b) during August, 1987. Only the correlation in (a) is significant (t-test; $p<0.01$).

a


y = .189x + .002 R-squared: .609
AWI
.055
.05
.045
.04
.035
.03
.025
.02
.015
.08
.1
.12
.14
.16
.18
.2
.22
.24
GSI

b

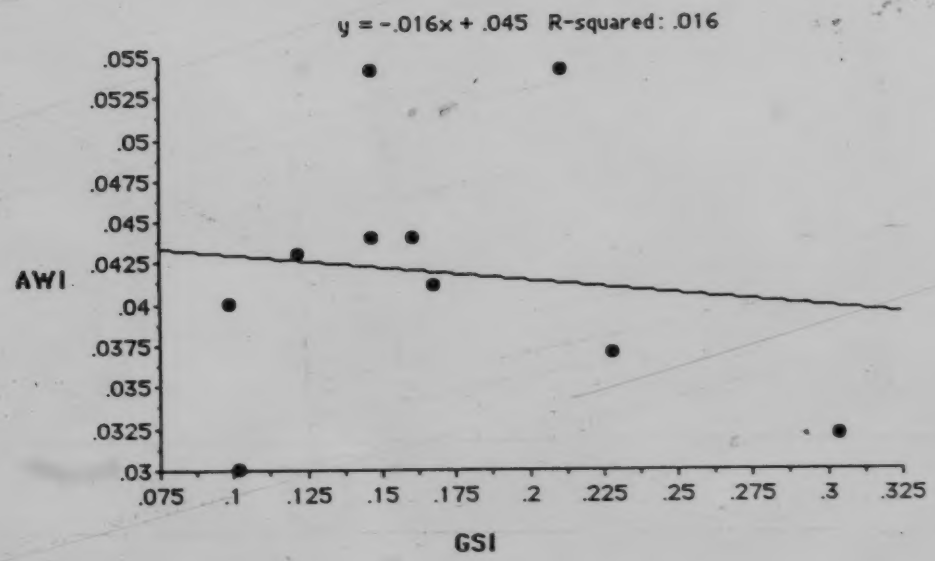
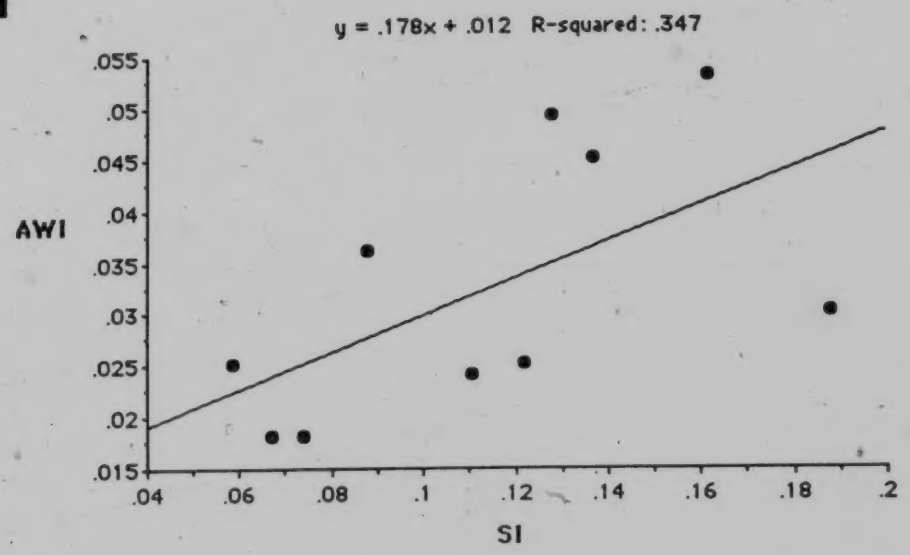

y = -.016x + .045 R-squared: .016
AWI
.055
.0525
.05
.0475
.045
.0425
.04
.0375
.035
.0325
.03
.075
.1
.125
.15
.175
.2
.225
.25
.275
.3
.325
GSI

FIGURE 19.

Relationship between AWI and SI in males sampled at NM (a) and FM (b) during August, 1987. Neither correlation is significant.

a


y = .178x + .012 R-squared: .347
AWI
.055
.05
.045
.04
.035
.03
.025
.02
.015
.04
.06
.08
.1
.12
.14
.16
.18
.2
SI

b

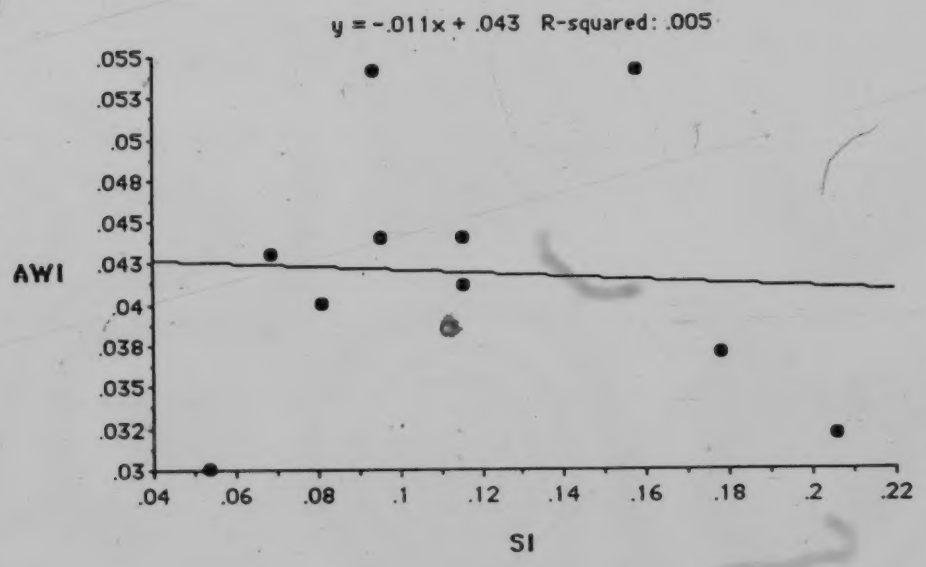
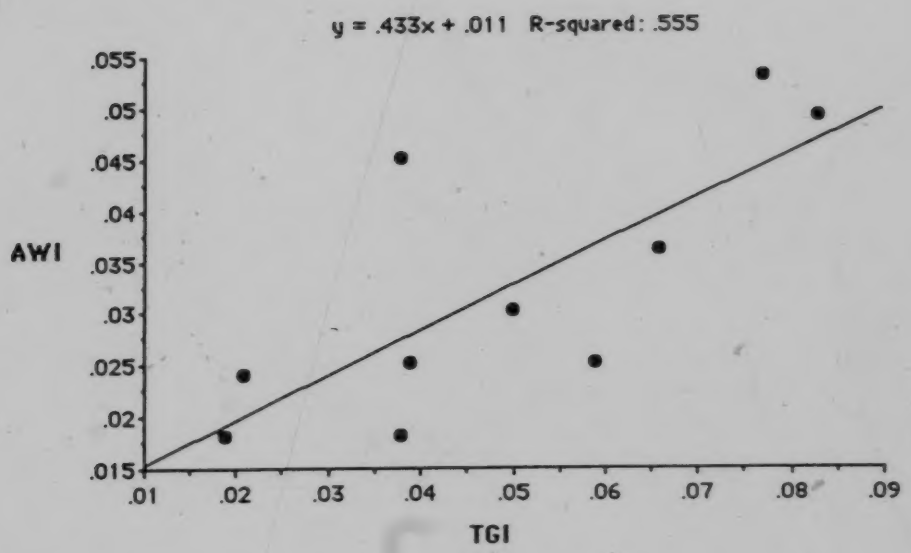

y = -.011x + .043 R-squared: .005
AWI
.055
.053
.05
.048
.045
.043
.04
.038
.035
.032
.03
.04
.06
.08
.1
.12
.14
.16
.18
.2
.22
SI

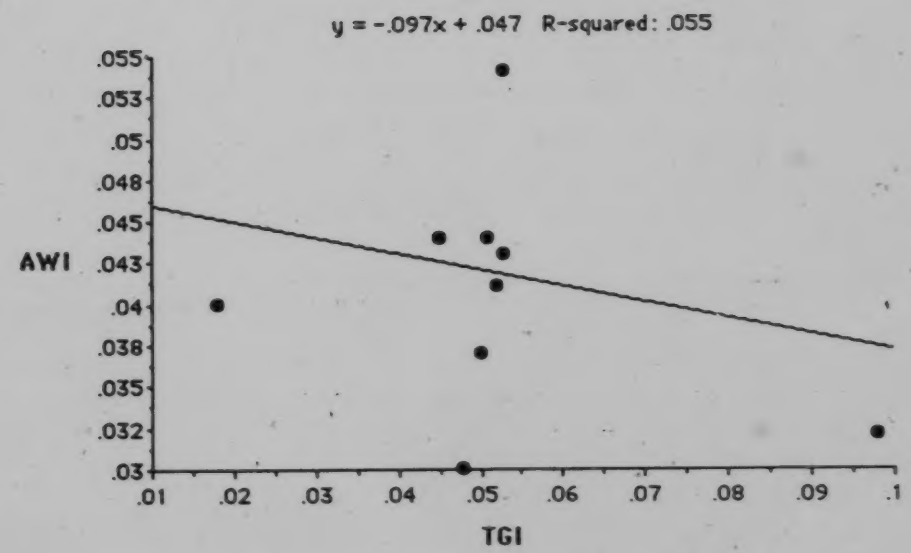
FIGURE 20.

Relationship between AWI and TGI in males sampled at NM (a) and FM (b) during August, 1987. Only the correlation in (a) is significant (t-test; $p<0.02$).

a


y = .433x + .011 R-squared: .555
AWI
.055
.05
.045
.04
.035
.03
.025
.02
.015
.01
.02
.03
.04
.05
.06
.07
.08
.09
TGI

b


y = -.097x + .047 R-squared: .055
AWI
.055
.053
.05
.048
.045
.043
.04
.038
.035
.032
.03
.01
.02
.03
.04
.05
.06
.07
.08
.09
.1
TGI

E. Histochemistry:

Mucous goblet cells were strongly PAS-positive, indicating the presence of neutral glycoproteins, whereas serous cells generally had weak PAS responses (Plate 5).

Mucous goblet cells exhibited strong aldehyde-fuchsin staining indicating sulphated glycoproteins (Plate 6). Serous cells picked up the orange G (an acid dye) very strongly, suggesting that their contents are acidophilic, a trait typical of proteins.

The mercuric bromphenol blue method is a widely used procedure for demonstrating proteins which stain a clear blue. Serous cells stained strongly (Plate 7), indicating that a major component of their secretory products must be proteinaceous. Mucous cells remained largely unstained.

PLATE 5.

AFF epidermis sampled at full moon (PAS & HE; 400x; 80A filter). M, mucous goblet cell; S, serous cell. Scale represents 20 μm.

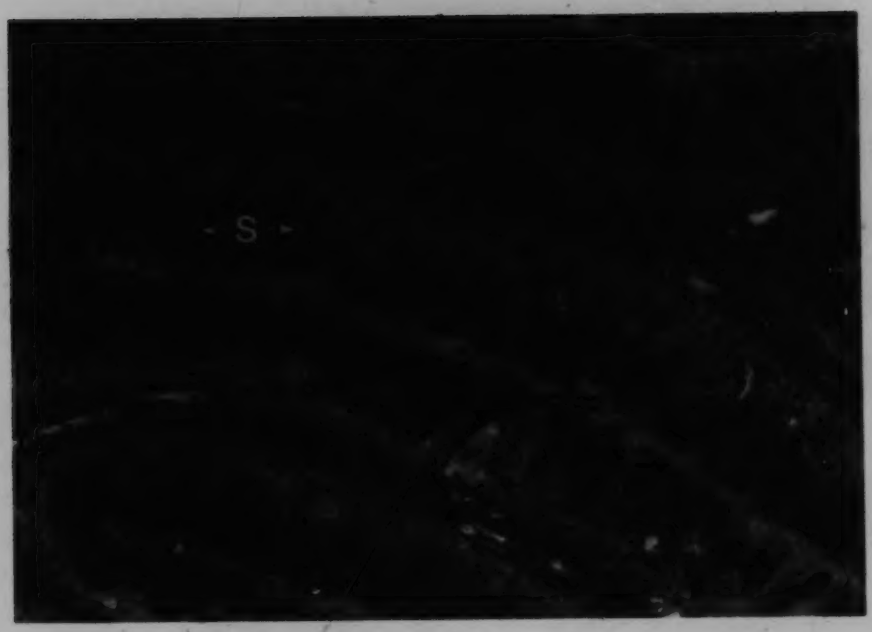

S

PLATE 6.

AFF epidermal tissue sampled at full moon and stained with aldehyde-fuchsin and orange G counterstain (400x). M, mucous goblet cell; S, serous cell. Scale repesents 20 μm.

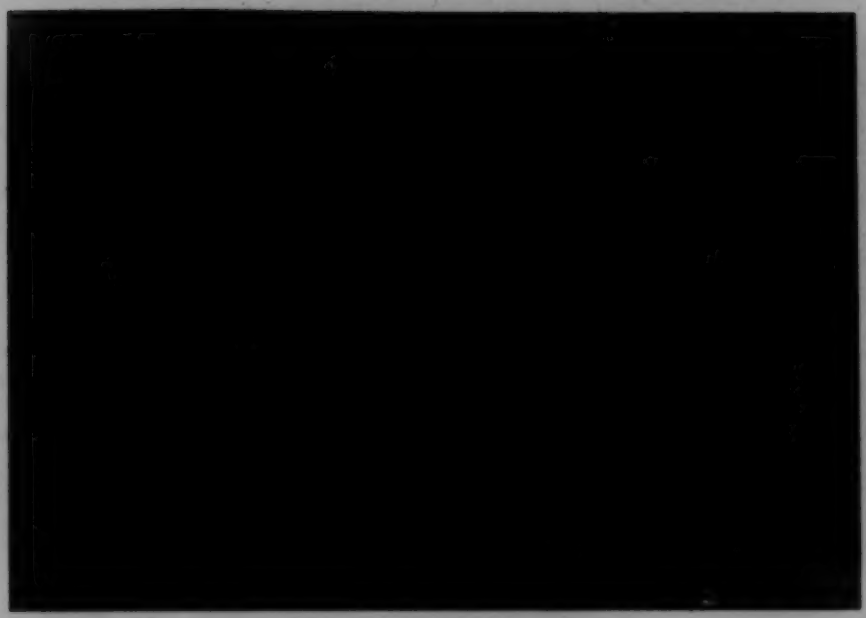


PLATE 7.

AFF epidermis sampled at full moon and stained with mercuric bromphenol blue (400x; ND 2 filter). M, mucous goblet cell; S, serous cell. Scale represents 20 μm.

S

F. Electron Microscopy:

Ultrastructural examination of the AFF epidermis revealed the presence of a fourth cellular component, the superficial epithelial cell (Plate 8), located at the surface of the epidermis such that the cuticle overlies it. As is known for other teleosts (Whitear, 1970), the cuticle is probably secreted by the superficial epithelial cells. These cells (6 to 8 μm diameter) appear flattened with microridges (or micropapillae) at their surfaces. The cytoplasm is filled with numerous secretory vesicles (Plate 9) which are moderately electron-lucent and were sometimes observed passing through the cell-membrane. Some ribosomal endoplasmic reticulum (rER) and Golgi systems were apparent (Plate 9).

Serous cells occur just below superficial epithelial cells (Plate 8, 10). The nucleus is basal (Plate 10) with very extensive and active rER and Golgi systems in the supranuclear region (Plate 11). There does not appear to be any peripheral displacement of these organelles. The most prominent feature of these cells is the abundance of membrane bound, electron-dense granules (0.4 to 1.0 μm diameter) in the supranuclear region. These granules exhibit two substructural patterns, both of which occur within the same cell. The first is a precise arrangement of concentric whorls (Plate 9), similar to the myelin pattern described in many cells from various organisms (see review by Ruby and Webster, 1972). The second is more typical of the substructure seen in other fish secretory cell granules, with the secretory

PLATE 8.

Electronmicrograph of AFF epidermal tissue, showing surface layers. A, cuticle; B, superficial epithelial cell; C, microridges; D, secretory vesicles; E, ribosomal endoplasmic reticulum (rER); F, serous cell; G, membrane bound granules; H, intercellular spaces. Scale represents 1 μm.

A
F
H
G
H

PLATE 9.

Electronmicrograph from surface layers of AFF epidermis. A, superficial epithelial cell; B, cuticle; C, microridges; D, secretory vesicles; E, membrane bound granule with "myelin-like" pattern; F, membrane bound granule with particulate substructure; G, intercellular space with empty membrane bound granule; H, Golgi system; I, rER. Scale represents 1 µm.

C
B
C
H
D
A
G
E
F
E

PLATE 10.

Composite electronmicrographs of AFF, showing different layers of epidermal tissue. A, superficial epithelial cell; B, serous cell; C, membrane bound granules; D, nucleus of serous cell; E, basic epithelial cell; F, nucleus of basic epithelial cell; G, secretory vesicles of basic epithelial cell; H, basement membrane; I, dermal or connective tissue; J, cuticle. Scale represents 1 μm.

A
B
B
C
E
G
F

PLATE 11.

Electronmicrograph of supranuclear region of a serous cell. A, nucleus; B, rER; C, Golgi system; D, granule in process of formation; E, intercellular space. Scale represents 1 μm.

D
C
E
C
C
B

product consisting of fine particluate matter. Granular forms intermediate between the two are also evident (Plate 12).

Basic epithelial cells have very large nuclei so that most of the organelles are perinuclear (Plate 10). There is a moderate amount of rER present and some Golgi systems. Electron-lucent secretory vesicles are common and some which are still membrane-bound but devoid of contents are found in spaces between cells (Plate 10).

Although some shrinkage is inevitable during preparation of the tissue, this is probably not enough to account for all the spaces observed between cells. Numerous empty, membrane bound, yet empty vesicles and granules commonly occur within these spaces. Further, the presence of cell-membrane projections around these spaces would indicate that these spaces are a natural attribute of the epidermis. The integrity of the superficial layer is intact; no apparent spaces occur between these cells.

No mucous cells were observed in this tissue sample.

PLATE 12.

Electronmicrograph of serous goblet cell. A, rER; B, Golgi systems; C, granule with "myelin-like" pattern; D, granule showing particulate substructure; E, granule with substructure intermediate between two types. Scale represents 1 μm.

C
A
E
B
D

G. Androgen Treatment Experiments:

Androgen-treated fishes had larger AFFs than controls (Plate 13a-c). These differences were significant for AAI values but not for AWI values (Fig. 21a,b).

There were marked differences in the AFF epidermal components among the three sets of specimens. There were no mucous cells in any of the specimens examined. Differences were most apparent in conditions and densities of basic epithelial cells and serous goblet cells.

The basic epithelial cell layer was thickest in control specimens (Fig. 22a). 11-ketotestosterone (KT)-treated fish had an epithelial cell layer thickness comparable to that in fishes collected over the FM and FQ phases while testosterone (T)-treated fish were similar to specimens collected over LQ and NM phases (Fig. 22a,b).

There were no serous cells in control specimen AFFs (Plate 14a). Serous cells in KT-treated fish were significantly taller and wider than those in T-treated fish (Fig. 23a and 24a; Plate 14b,c). Serous cell heights in KT-treated fishes were most similar to those observed in fishes collected at FM (Fig. 23a,b). The widths of cells from fishes treated with this androgen are comparable to those in fishes collected at FM (Fig. 24a,b). The granular composition of serous cells observed in androgen-treated specimens is similar to that observed in fishes collected outside spawning periods.

PLATE 13.

Effects of androgens on AFF size. All sections stained following the PAS procedure with Harris hematoxylin counterstain (100x; CC10C & CC20C filters). (a) Cross-sectional view of AFF from a control specimen (SL = 72.0 mm). (b) Cross-sectional view of AFF from a testosterone-treated specimen (SL = 71.1 mm). (c) Cross-sectional view of AFF from an 11-ketotestosterone-treated specimen (SL = 72.1 mm). Scale represents 0.2 mm.

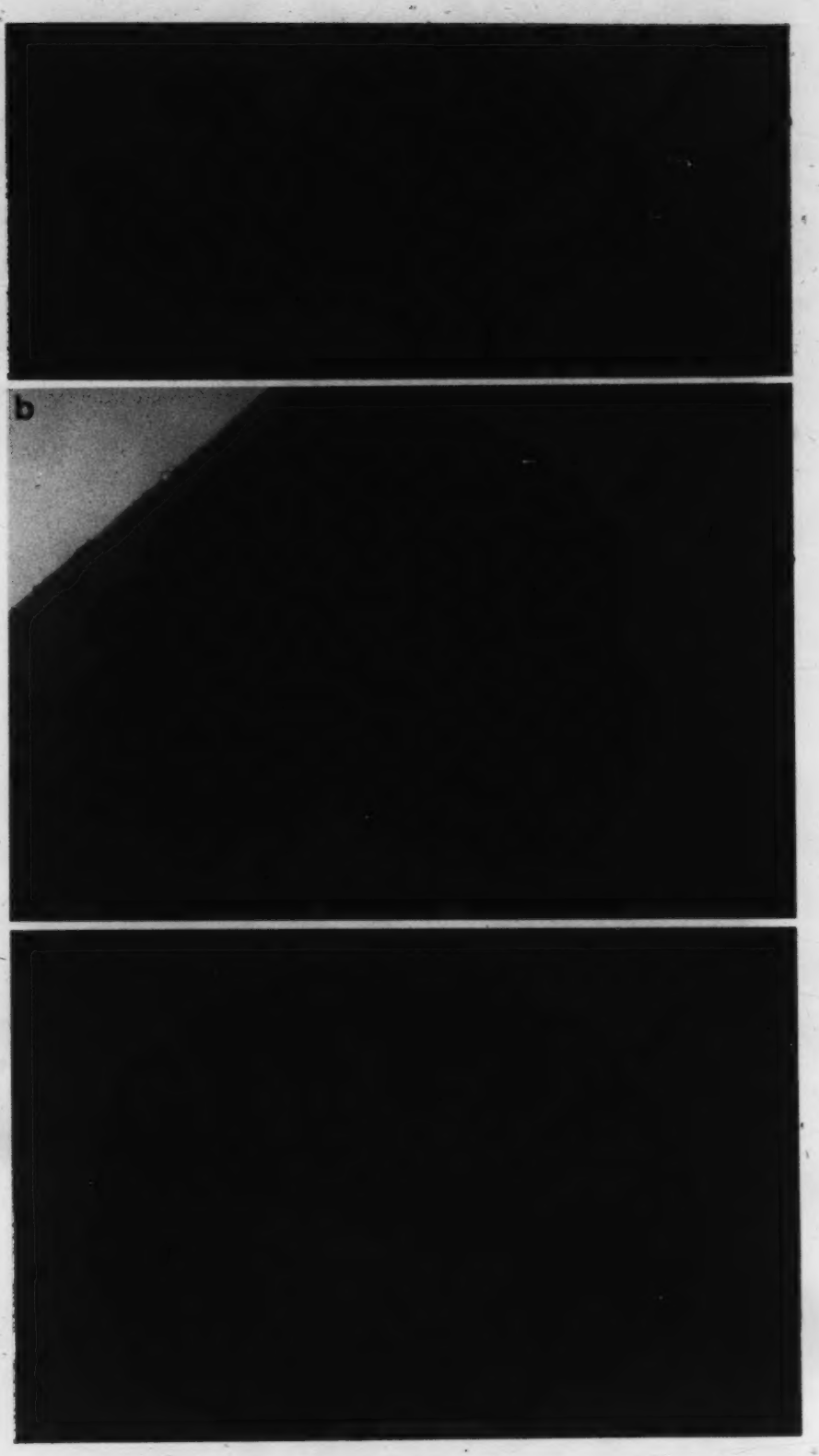
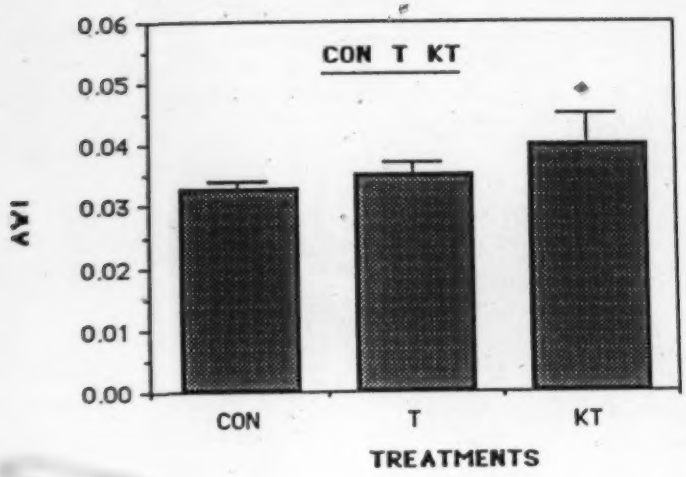

b

FIGURE 21.

Effects of androgens on AFF size. (a) AWI values. (b) AAI values. Vertical bars represent standard errors about the mean. (Con = saline control; T = testosterone; KT = 11-ketotestosterone). Groups not underlined by the same line are significantly different ($p<0.05$). N = 10 for each treatment.

a


CON T KT
0.06
0.05
0.04
0.03
0.02
0.01
0.00
AVI
CON
T
KT
TREATMENTS

b

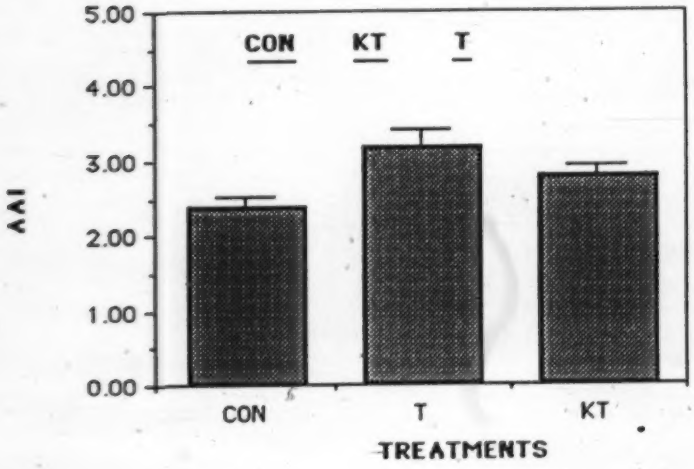

CON KT T
5.00
4.00
3.00
2.00
1.00
0.00
AVI
CON
T
KT
TREATMENTS

FIGURE 22.

AFF basic epithelial cell layer thickness (μm; ±SEM). (a) Effects of androgens. (Con = saline control; T = testosterone; KT = 11-ketotestosterone; n = 10 for each treatment group). (b) Data from the August, 1987 histometric sampling study are included for comparison. (n = 10 for each lunar phase). Groups not underlined by the same line are significantly different ($p<0.05$).

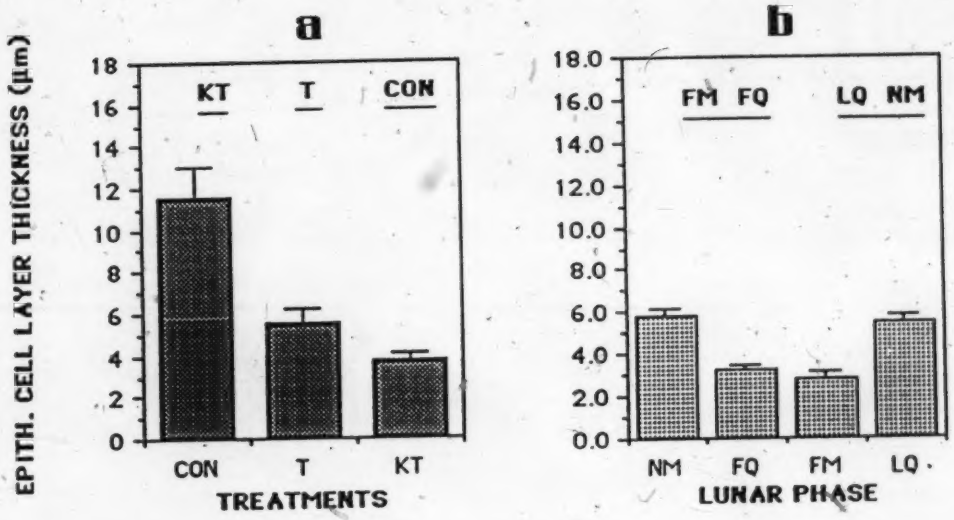

a
b
EPITH. CELL LAYER THICKNESS (µm)
18
16
14
12
10
8
6
4
2
0
KT
T
CON
CON
T
KT
TREATMENTS
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
FM FQ
LQ NM
NM
FQ
FM
LQ
LUNAR PHASE

PLATE 14.

Photomicrographs of AFF epidermis. All tissue sections stained according to the PAS method with HE counterstain (400x; CC10C & CC20C filters). (a) AFF epidermis from a control specimen (SL = 72.0 mm). (b) AFF epidermis from a testosterone-treated specimen (SL = 71.1 mm). (c) AFF epidermis from an 11-ketotestosterone-treated specimen (SL = 72.1 mm). Scale represents 20 μm.

FIGURE 23.

AFF serous cell height (μm; ±SEM). (a) Effects of androgens.(T = testosterone; KT = 11-ketotestosterone). Note that the control group specimens did not have any serous cells. N = 10 for each treatment group. (b) Data from the August, 1987 histometric sampling study are included for comparison (n = 10 for each lunar phase). Groups not underlined by the same line are significantly different ($p<0.05$).

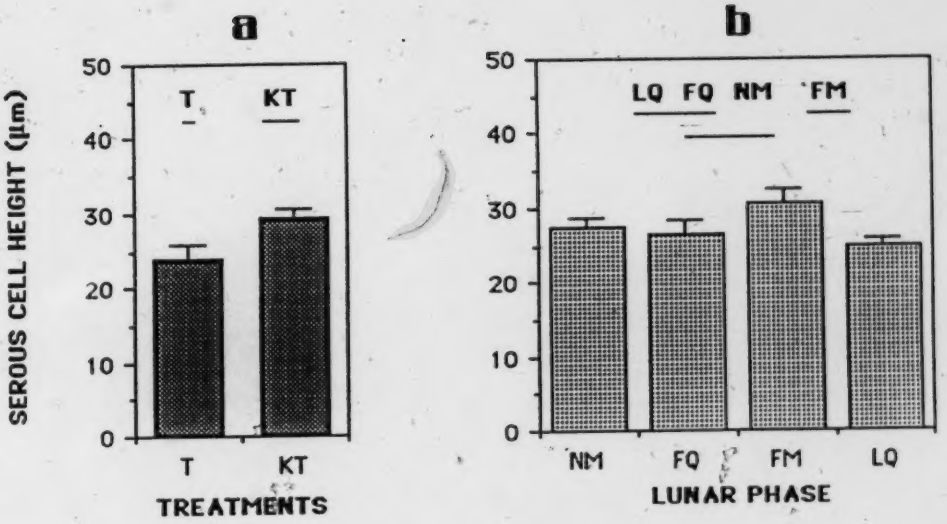
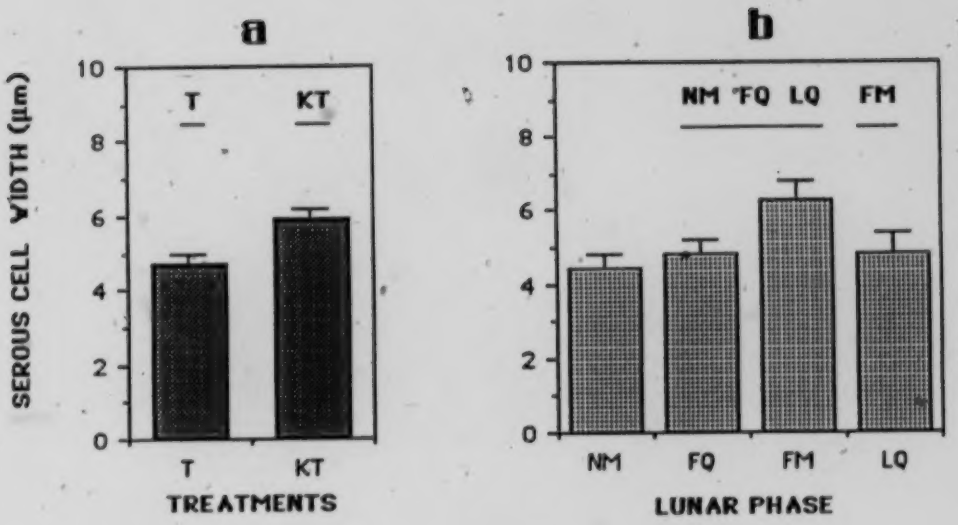

a
b
SEROUS CELL HEIGHT (µm)
50
40
30
20
10
0
T
KT
TREATMENTS
LQ FQ NM FM
NM
FQ
FM
LQ
LUNAR PHASE

FIGURE 24.

Serous cell width (μm; ±SEM). (a) Effects of androgens.(T = testosterone; KT = 11-ketotestosterone; n = 10 for each treatment group). (b) Data from the August, 1987 histometric sampling study is included for comparison (n = 10 for each lunar phase). Groups not underlined by the same line are significantly different ($p<0.05$).

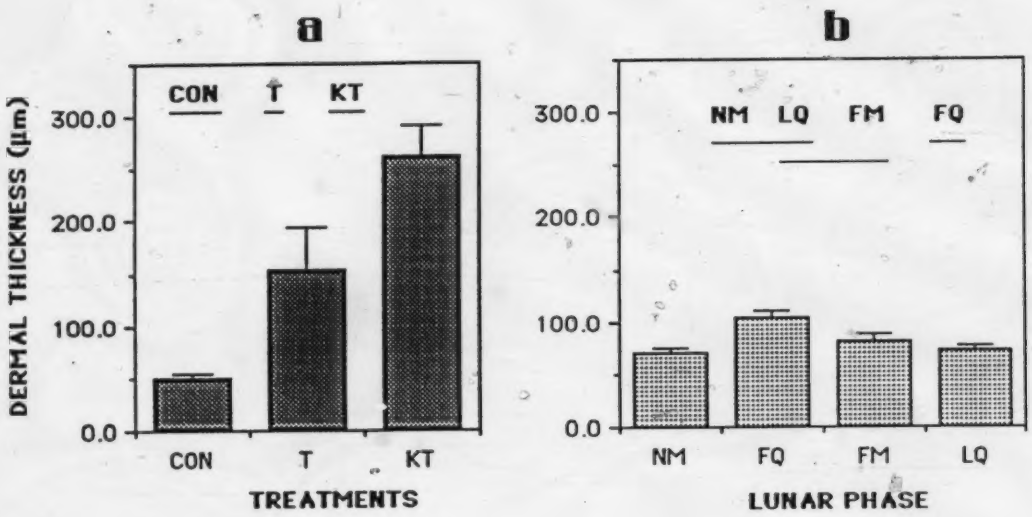

a
SEROUS CELL WIDTH (µm)
10
8
6
4
2
0
T
KT
T
KT
TREATMENTS
b
NM FQ LQ
FM
10
8
6
4
2
0
NM
FQ
FM
LQ
LUNAR PHASE

Differences also occur in the dermal components of AFFs of specimens from these experiments. Androgen-treated fishes had significantly thicker dermal components than controls, with this proliferation being greatest in KT-treated specimens (Fig. 25a). The dermal layer in control fish AFFs is comparable to that in fishes from the sampling study (Fig. 25a, 25b).

The mean GSI of T-treated fish (0.153±0.020) was significantly ($p<0.05$) higher than those of KT-treated (0.102±0.010) or control (0.102±0.009) fish.

FIGURE 25.

AFF dermal tissue thickness (μm; ±SEM). (a) Effects of androgens. (Con = saline control; T = testosterone; KT = 11-ketotestosterone; n = 10 for each treatment group). (b) Data from the August, 1987 histometric sampling study are included for comparison (n = 10 for each lunar phase). Groups not underlined by the same line are significantly different ($p<0.05$).


a
b
CON
T
KT
NM
LQ
FM
FQ
300.0
200.0
100.0
0.0
DERMAL THICKNESS (µm)
CON
T
KT
TREATMENTS
NM
FQ
FM
LQ
LUNAR PHASE

V. DISCUSSION

The present investigation has shown that the AFF in O. atlanticus is a complex structure which undergoes periodic cytological and cytochemical changes closely associated with the lunar spawning cycle. Most of these changes are probably mediated by androgens. These findings and their implications are considered below.

Gross morphology, cytomorphology, and cytochemistry of the AFF :

This study has shown that the AFF in O. atlanticus is morphologically comparable to similar structures reported in other blenniids, although important differences are also observed. With the possible exception of Xiphasia spp. (a highly modified member of the tribe Nemophini), all sexually mature male blenniids possess anal fin modifications which can be categorized on the basis of their shape and on the number and type of supporting element involved: i.e. whether they are found on the anal fin spines or rays or both (Porter, 1979).

The most common arrangement is where both spines and rays bear glandular modifications but the configuration of the two sites is different. This pattern is widespread among the Blenniini and Nemophini (Porter, 1979) and is seen for e.g. in Blennius pavo (Patzner and Seiwald, 1987) and B. incognitus (Peterson, 1984).

The least common and simplest pattern, where only the anal fin spines are modified, occurs in only a few species of the Blenniini and Salariini. This is the arrangement found in O. atlanticus and also in O. steindachneri (Porter, 1979), a Pacific species, both of the Salariini.

There appear to be two distinct patterns to these modifications on the anal fin spines. The first is typified by a bundle of microspheres around the spines (Exallias brevis, Porter, 1979; Blennius gattorugine, Kotrschal and Goldschmid, 1983). The second and apparently more widespread type has a more elongated and folded arrangement (O. steindachneri, Porter, 1979; B. cristatus, and B. rouxi, Peterson, 1984; B. pavo, Patzner and Seiwald, 1987b; O. atlanticus). Even among anal spine modifications of the folded type there are variations. For instance, there can be differential development of these AFFs between spines among species. In most species studied (E. brevis, Porter, 1979; B. rouxi and B. cristatus, Peterson, 1984; B. pavo, Patzner and Seiwald, 1987b), both AFFs are of comparable size, whereas in O. atlanticus the first AFF is larger and more developed than the second. Furthermore, Patzner and Seiwald (1987b) described proximal secretory canals in the anal fin pads of B. pavo which they reported to vary in diameter with the spawning activity of the males. There was no evidence, in the present study, of comparable structures in the AFFs of O. atlanticus.

Porter's (1979) use of the term anal fin glands implies that all the blenniid anal fin spine modifications are glandular in nature. However, R. L. Smith (1974) described spatulate, non-rugose pads lacking a glandular character in a number of blenniids which he suggests function in anchoring these fishes to the substratum. Notwithstanding, this latter study was limited to comparative gross morphology and glandular elements cannot be dismissed without corroborative histological examinations.

Other members of the Blennioidea, specifically some representatives of the Clinidae (e.g. **Gilloblennius decemdigitatus**) also bear swellings on the spines and rays of the anal fins in males (Porter, 1979). Such structures apparently are not restricted to blennioids as they have been reported in males of **Acanthoclinus quadridactylus** (Perciformes, Acanthoclinidae; Porter, 1979). Organs similar to the AFFs of blenniids have also been observed in some siluroid fishes (**Paraplotosus, Plotosus, Cnidoglanus** and **Oloplotosus**; Blüm, 1972), although no details of their structure or function were discussed.

These blenniid features are distinct from the epidermal breeding tubercles or pearl organs which develop, usually in the male, in a variety of other fish, notably the Salmonidae, Plecoglossidae, Osmeridae, Retropinnidae, Belonidae, Cyprinidae, Catostomidae and Cottidae (Wiley and Collette, 1970) since these latter structures are largely keratinized tissue (Whitear, 1986) and not glandular.

Among fishes, examples where epidermal glands are present only in males are rare and include the caudal glands of the characid subfamily Glandulocaudinae (Nelson, 1964; Atkins and Fink, 1979), and the sunken patches of epidermis opening by slits on the head of **Arapaima gigas** (Lüling, 1964). Breeding male fathead minnows, **Pimephales promelas** have distinct dorsal and head pads which have high densities of mucous cells (Smith and Murphy, 1974) and could be placed within the above category except that these structures consist of both epidermal as well as conspicuous dermal components.

The AFF epidermis in O. **atlanticus** is more complex than the much less developed fin epidermis of most other fishes. Bullock and Roberts (1974) stated that in most fish, the epidermis over fins and tail is often qualitatively different from that of the body in that it is much thinner, usually lacks the more specialized cells and where there are two types of mucous cell elsewhere only one of the types will be found in these regions. The histology of the anal fin swellings in **Acanthoclinus quadridactylus** is distinct from that of blenniids, particularly in the epidermis where no glandular elements are found (Porter, 1979). Tissue organization of similar structures in **Gilloblennius decemdigitatus** is more similar to that observed in blenniids (Porter, 1979) although it is uncertain if elongated cells within the epidermis are in fact secretory.

In spite of the variety among blenniids in anal fin spine modifications reported at the gross anatomical level, it appears that the basic arrangement of tissues is similar. In all cases, the spine is embedded in connective tissue (both loose and dense forms) which in turn is covered by a layer of epidermal tissue (Porter, 1979). While Porter's (1979) investigations indicated that the epidermal organization was very similar among blenniids, other studies, including the present one, demonstrate interspecific differences.

The arrangement of basic and superficial epithelial cells among the various blenniids is apparently similar. It is interesting that in the present study the superficial epithelial cells (which were mistaken for the cuticle in the histological study) were discernible only upon ultrastructural examination of the epidermis. The cuticle is still present but is much thinner than originally thought. It appears that the AFF epidermal tissue in this species is extremely dense. Thin sections in the order of 2 μm were required for histology before serous goblet cell configurations were visible whereas in all other studies even 10 μm sections seemed adequate. This is consistent with the situation in the present electron microscopy study where ultra-thin sections in the silver-gray range (<80 nm) had to be used before acceptable resolution was achieved.

Porter (1979) found tall cylindrical cells with basal nuclei in the AFF epidermis of various species. These cells

were arranged around an amorphous core and she termed these barrel-shaped forms "microglands". Peterson (1984) found both mucous and tall, cylindrical, protein-secreting cells in the AFF epidermis of several **Blennius** spp. Such cells were also observed in the dorsal and anal fin epidermis while a third cell-type (knob-like cells) were largely restricted to these median fin regions only. In some cases, cylindrical cells were completely absent, but where they were present, Peterson (1984) was able to recognize different categories. In some species (e.g., **B. galerita**), 8 to 10 cylindrical cells are clumped together throughout the epidermis with each group sharing a single secretory canal, while in others (e.g., **B. incognitus** and **B. cavenai**), 3 to 4 cells shared a single opening in the superficial epithelial layer opening to the exterior. The patterns in **B. cristatus** and **B. velifer** (Peterson, 1984) are similar to that found in **O. atlanticus**, with the cylindrical cells occurring more or less uniformly throughout the epidermis without clumping and with no apparent canals or breaks in the superficial epithelial layer leading to the exterior. In both the present histological and electron microscopical studies, there was no evidence of any break in the superficial epithelial cell layer or of any common canals or ducts leading to the exterior. The presence of any such structures cannot be excluded since I did not examine the entire surface of any one AFF microscopically. However, the presence of large flask-shaped mucous goblet cells with their "necks" leading to the surface indicates

that there must be some break in the superficial layers. Mucous goblet cells were reported in variable numbers in many different AFF epidermal tissues (B. incognitus and B. canevai, Peterson, 1984; B. pavo, Laumen et al., 1974) but not in others (B. galerita and B. cristatus, Peterson, 1984).

The aldehyde-fuchsin procedure was used to differentiate mucous goblet cells from serous cells and also served to demonstrate sulphated glycoproteins within the AFF goblet mucous cells. Nevertheless, I did not attempt any further characterization of the chemical nature of these cells. Most workers agree that glycoproteins are a major component of fish mucus. It is also agreed that the biochemistry of fish mucus varies with species, but such variations cannot, at present, be correlated with habit or with habitat (Mittal et al., 1979). There is variation in the physical/chemical properties of mucus from even closely related species (Rosen and Cornford, 1971). Peterson (1984), in detailed analyses of the mucous cells from various Blenniidae found wide differences among genera and even between congeneric species. The type of mucus secreted by individuals is also affected by environment (Solanki and Benjamin, 1981) and stage in the life history (Blackstock and Pickering, 1982). Furthermore, histochemistry by its very nature is essentially limited to identifying secretory products of cells only while they are still contained within secretory granules inside the cell. Upon release to the surface, such secretions usually aggregate or cross-link with other types of secretions to

produce the final functional product (Reid and Clamp, 1978). Hence, data on the chemical products of various cells should be treated with circumspection.

The serous cells observed in the AFF share a cytomorphology common to fish goblet cells in general. Hence, the term "serous goblet cell" will be used to describe the cells in the present study. Such exocrine unicellular glands have basally located nuclei, contain much ribosomal endoplasmic reticulum and prominent Golgi systems, and usually have their secretions in the form of membrane-bounded globules which normally remain intact until shortly before discharge (Whitear, 1986). Kotrschal and Goldschmid (1983) described the cylindrical glandular cells observed in the median fins and the AFF in 10 species of **Blennius** as being a type of goblet cell which is consistent with the description presented in the current study. However, none of the other studies on the Blenniidae have examined such cells by TEM, making comparisons difficult. Similar cells have been reported, although not always confirmed by TEM, in numerous other fishes: **Chaenogobius castanea** (Sato and Kurotaki, 1964); **Tetraodon fluviatilis** (Mittal and Banerjee, 1976); **Mastacembalus erythrotaenia** (Zaccone, 1982); **Latimeria** (Whitear, 1977); **Trigla lucerna**, as well as in the epidermis under the operculum of labrids and scarids (Whitear, 1986). The acidophilic granular cells occurring sporadically in the epidermis of salmonids (Blackstock and Pickering, 1980) are actually serous goblet cells (Whitear, 1986).

The current ultrastructural study of serous goblet cells has demonstrated a unique concentric-patterned packaging within many secretory granules. At present, there have been no reports of any comparable substructural elements in other fish secretory cells. In fact, there are no published reports in the literature of any similar structures. Such packaging does resemble the membranous whorls, known as myelin figures, reported in a wide variety of diverse cells. These have been observed not only during the normal processes of cell degeneration (de Duve, 1969) but are also associated with a variety of other factors such as cell stress, cell pathology, changes in protein nutrition, etc. (see review by Ruby and Webster, 1972). Such myelin-like lamellar bodies have even been reported in the TG cells of B. pavo (Seiwald and Patzner, 1987). However, whereas these substructures in B. pavo are membrane-bound, the packaging in the redlip blenny serous goblet cells definitely is not. The significance of such substructures within these cells is unknown.

Ultrastructural examination of the AFF epidermis demonstrated numerous intercellular spaces. Hence, the epidermis appeared "sponge-like" in composition. Such widely dilated intercellular gaps are often observed in teleost epidermis and there is structural evidence that the contents of secretory cells are released into these spaces (Henrikson and Matoltsy, 1968). It is probable, then, that the AFF epidermal intercellular spaces probably hold the secretory products from the serous goblet cells prior to their release

from the AFF.

The secretions of serous goblet cells are generally proteinaceous in nature, and this is also true of the serous goblet cells reported in the current study. Kotrschal, Weisl and Goldschmid (1984) showed that the cylindrical goblet cells in both median fins and in anal fin modifications in various Blenniidae secreted high amounts of proteinaceous compounds as well as acid glycoproteins. The cylindrical cells reported by Peterson (1984) had neutral glycoproteins and acidophilic sulphated glycoproteins although the contents varied between genera and species. In B. cristatus, which has a cylindrical cell arrangement similar to that of the redlip blenny, the cylindrical cells have high levels of general proteins as well as some polysaccharides (Peterson, 1984). While neutral glycoproteins were absent in serous goblet cells in the present study, it is possible that acid glycoproteins (which were not tested for) were present. Little is known, at present, regarding the functional significance of protein secretions from serous goblet cells, although it is generally accepted that very different functions might apply in different species and in different situations.

Relationship of the AFF to the spawning cycle :

The results of this study are consistent with earlier documentations (Marraro, 1978; Labelle, 1982; Marraro and Nursall, 1983; Côté, 1987) that O. atlanticus has an extended

breeding season with a lunar periodicity in spawning activity. Spawning is known to occur from February to August (Marraro, 1978) although peak numbers of fish spawning have been reported in spring (Labelle, 1982). There are no reports of lunar spawning cycles in European blenniids which have been shown to display annual reproductive cycles with peak spawning occurring over several months, typically in the summer (B. pholis, Shackley and King, 1977; B. pavo, Patzner, 1983). Although multiple spawning has been reported in B. pholis (Quasim, 1956; Shackley and King, 1977), reproduction is limited to a single period during the year. Hence different states of quiescence and increased activity are readily observable within the gonads in such species. Patzner and Seiwald (1987a) showed that GSI in male B. pavo remained low throughout most of the year but peaked dramatically at the start of the spawning season. The changes in GSI of male O. atlanticus show no such relationship to the lunar spawning cycle although the lowest values were consistently recorded at FQ i.e. the week preceding spawning. Differences in seminiferous tissue over the lunar spawning cycle in the redlip blenny were consistent with those reported in B. pavo (Patzner and Seiwald, 1987a), with maximal sperm production occurring just before and during peak periods of spawning. However, where these workers were able to document definite spawning season peaks in both TG size and TG cell size, no such patterns were observed in O. atlanticus in the present investigation. It is possible, given the high frequency of

spawning in this species, that there is asynchronous activity of the TG cells such that not all these cells are active at the same time. This could possibly account for the complicated GSI profile obtained here. The possible role of the TG is discussed in the section on endocrine mediation.

Many marine organisms have been reported to undergo monthly spawning cycles (Luckhurst and Luckhurst, 1977; Johannes, 1978). Lunar spawning in the redlip blenny is probably important in maintaining reproductive synchrony between the sexes. A variety of hypotheses exist attempting to explain lunar spawning activity in reef fishes (Allen, 1972; Johannes, 1978; Lobel, 1978; Pressley, 1980). Among the most widely accepted are that moonlight might help positively phototactic larvae avoid benthic predators (Allen, 1972; Moyer, 1975) and also that lunar spawning cycles minimise larval and egg predation by facilitating their transport offshore away from reef associated planktivores (Johannes, 1978). Côté (1987) postulated that peak hatching in redlip blennies should occur only at ebbing tides and at maximum moonlight intensity if larval survival is to be maximised.

The AFF of sexually mature O. atlanticus displays a clear lunar periodicity, with maximal development coinciding with peak periods of spawning at full moon. Such changes associated with reproductive periods have also been shown in B. cristatus (R. L. Smith, 1974) and B. pavo (Patzner and Seiwald, 1987b) as well as other Blennius species (Zander, 1975; Papaconstantinou, 1979). None of these other studies,

however, have described specific changes within the AFF tissues associated with spawning.

The present investigation demonstrates that AFF developmental changes are due mainly to changes within the epidermis, including an overall thickening of this tissue, specifically by the proliferation of mucous goblet cells and by an increase in size and secretory activity of serous goblet cells. Basic epithelial cells, on the other hand, were most numerous outside spawning periods. Unlike higher vertebrates, where the basal epidermal cells constitute a germinal layer, in fishes, mitotic activity is detectable throughout the epidermis although commonest in the deeper layers (Whitear, 1986). Thus, basic epithelial cells in the AFF epidermis probably give rise to the secretory cells by mitotic differentiation. If this is the case, then there should be an inverse relationship between secretory cell and basic epithelial cell densities. Indeed, Laumen et al. (1974) described only epithelial cells 4 to 8 layers thick as well as some mucous cells in the AFF of B. pavo but failed to find the cylindrical glandular cells described by Eggert (1931) and Peterson (1984). It is likely that the Laumen et al. (1974) study, which examined few specimens, encountered only sexually inactive fishes, within which non-mucoid glandular elements may not have developed. Several fishes within the current study had unswollen AFFs and also lacked glandular elements of any nature within the AFF epidermis.

European blenniids do not possess such a dense packing of AFF epidermal tissue as is seen in O. atlanticus. This might be linked to the differences in reproductive activity of these fishes. European blenniids, with their shorter breeding seasons, would probably not use their AFFs for much of the year, and hence might not need the concentrations of epidermal secretory cells required by the redlip blenny. An extended breeding season in O. atlanticus, in which a number of males have been observed spawning during every lunar cycle (Côté, 1987), conforms with this observation.

The thickness of fish epidermis generally varies with species, age, position on body and in many cases, stage of the reproductive cycle (Bullock and Roberts, 1974). Although seasonal changes in teleost epidermis are not universal, they have been reported in some members of the families Catostomidae and Cyprinidae (Smith, 1976), in Salmo trutta (Pickering, 1977), Salmo salar (Wilkins and Jancsar, 1979) and the winter flounder Pseudopleuronectes americanus (Burton and Fletcher, 1983). Often sexual dimorphism in integumentary tissues is exhibited by some teleosts only during periods of spawning (e.g. in anadromous Salmo trutta trutta, Stoklosowa, 1970; in several species of Catostomidae and Cyprinidae, Smith, 1976; and in the threespined stickleback, Gasterosteus aculeatus, Burton, 1979). Most of these changes involve simple thickening of the entire epidermal layer, increased mucigenesis (proliferation of mucous cells) and increased epidermal melanophore density. Dorsal pad development in

Pimephales promelas occurs during the breeding season and consists mostly of changes in epidermal thickness, mucous cell number and especially in dermal connective tissue thickness (Smith, 1978).

Endocrine mediation of AFF development:

As in higher vertebrates, the skin of teleosts is an androgen target organ capable of androgen metabolism (Roberts, 1974; Pickering, 1975). The current investigation provides strong evidence that AFF development in the redlip blenny is androgen-mediated, with 11-ketotestosterone (11-KT) having a greater effect than testosterone (T). This is consistent with the observed lack of innervation in these structures, which indicates that their control is probably hormonal. Both dermal and epidermal tissues were affected but in different ways. Androgen-induced increases in the dermis were more pronounced than those observed over a natural spawning cycle.

Of the AFF epidermal cell types, serous goblet cells apparently were most sensitive to androgens, although a decrease in basic epithelial cell density occurred concomitantly with an increase in size and number of the serous cells. Serous goblet cell development in response to androgens was quantitatively similar to that observed in field samples. Changes in size comparable to those at FM were produced by 11-KT whereas goblet cell size in T-treated fish was closer to that in non-breeding wild males. Qualitatively, however, the density of granules within the serous cells of

androgen-treated fish was comparable to what was observed in serous goblet cells outside natural spawning periods, indicating that peak secretory activity had not been achieved despite androgen administration. This implies that androgen dosages used in these experiments might not have been high enough to match endogenous levels at peak periods of spawning. Alternatively, other untested hormones may also play important roles controlling the activity of these cells.

Epithelial cell layer thickness in 11-KT-treated specimens was comparable to that of field specimens at peak spawning periods whereas that of T-treated specimens more closely resembled non-breeding specimens. Control specimens showed a basic epithelial cell layer that was remarkably thicker than either wild or androgen-treated specimens but which also completely lacked glandular elements. It is possible that maintenance of serous and mucous goblet cells requires a certain level of circulating androgens or other hormones, and that the endocrine systems in these control specimens might have become depressed in captivity. Indeed, T-treated fish had significantly ($p<0.05$) higher GSIs than either control specimens or KT-treated fish. GSI values for these latter two groups were considerably lower than values observed in field samples, suggesting that testicular tissues had atrophied in captivity. It is not known why only the testes of T-treated specimens seemed unaffected nor whether the two testicular components were affected differently in the other groups.

Mucous cells were completely absent in all experimental specimens, suggesting either that their development within the AFF is not controlled by androgens, that the dosages used were insufficient to promote their development or that high levels of androgens might inhibit their development. In some teleosts, fish prolactin may maintain epidermal mucous cells (Ball, 1969) or even induce mucigenesis in some cichlids (Blüm, 1972) while ovine prolactin produced considerable epidermal thickening and mucigenesis in some euryhaline species (**Mugil auratus**) but especially in stenohaline fish (Blüm and Fiedler, 1972). Blüm (1972) found that both FSH and LH produce a decrease in number of epidermal mucous cells in male **B. sphinx** which could imply that androgenic steroid release may lower mucous cell densities. This would be consistent with the present findings.

Among blenniids, the only published study on the effects of hormones on the AFFs are for **B. sphinx** (Blüm, 1972) in which both LH and FSH-treated fishes showed maximal development of the AFFs. This development was associated with an increase in the diameter of unicellular "ducted" glands within the epidermis. These glands were not well characterized in Blüm's study but do not appear to be cytomorphologically similar to the serous goblet cells reported in the present study. Blüm also reported that these AFF size increases were not dose-related.

A number of other studies have documented the effects of androgens on teleost skin. Most of these hormonal changes

involve alterations in the thickness and complexity of the epidermis but interspecific differences exist. McBride and Van Overbeeke (1971) showed that androgens (such as 11-KT and 17α-methyltestosterone) induce significant increases in epidermal thickness and in number of mucous cells in male sockeye salmon (**Oncorhynchus nerka**), while estrogens have a similar but lesser effect on females. It is possible then, that androgen treatment had a general effect on epidermal tissues although this was not investigated.

The present finding that androgens stimulate AFF development is consistent with a number of other documentations of androgenic control of male secondary sexual characters in fish (Liley and Stacey, 1983). Smith (1974) found that methyltestosterone induced formation of both breeding tubercles and the dorsal pad in **Pimephales promelas**. This androgen increased both the connective tissue layer of the dorsal pad and the overall epidermal thickness and initiated a proliferation of mucous cells. Such changes were not observed in other portions of the skin.

The present investigation demonstrated that androgen-induced changes within the AFF, particularly in the thickness of the basic epithelial cell layer and in the size of serous goblet cells, are comparable with those observed in the natural spawning cycle. Thus, it is reasonable to conclude that androgens play an important controlling role on the AFF. The fact that 11-KT had more potent effects (with the exception of the effect on AAI) than T is not surprising

since the former is regarded as the principal androgenic steroid in teleosts (Liley and Stacey, 1983). At the present time, it is unknown why the androgen-induced proliferation in connective tissue in the AFF was much greater than that observed in the wild. Smith (1974) observed the reverse effect for fathead minnows; connective tissue thickness in the androgen-treated dorsal pad was not as great as in the natural state. He suggested that full development of this layer may require high levels of pituitary hormones as well as androgens.

Sexually immature male redlip blennies also possess AFFs (personal observation). The fact that these AFFs invariably lack a glandular epidermis (data not shown), is further evidence that glandular epidermis development requires the stimulus of androgenic steroids.

From the above considerations, it is apparent that AFF development in general and its epidermal configuration in particular might be useful indicators of endogenous androgen levels within this species.

Androgen production and metabolism in teleosts has been shown to occur not only in the testis and genital tract (see Fostier et al., 1987 for review) but also in interrenal tissue (Idler and Mcnab, 1967; Sufi et al., 1980; Schulz, 1986), in the skin (Ali et al., 1987) and in liver (Kime, 1978; Schulz, 1986) and blood cells (Schulz, 1986). The importance of some of these organs and tissues towards maintaining plasma androgen levels is controversial and

requires further study. Within the gonads, it is now accepted that Leydig cells (similar to those reported in other vertebrate classes) occurring within the interstitial tissue of testes are the major source of androgens in teleosts, although Sertoli cells, epithelial cells of the genital tract and even spermatozoa have also been implicated (see Fostier et al., 1987).

In the present study, Leydig cells were not observed within the seminiferous tissue of the redlip blenny testis. However, in view of the poor resolution of histological and even histochemical studies for detecting these cells, their absence cannot be corroborated without ultrastructural studies. Preliminary histochemical analyses (Sudan Black and Sudan Red tests) have demonstrated fairly intense sudanophilia within the testicular gland (TG) of the redlip blenny at peak spawning times (data not shown), suggesting an accumulation of lipids (typical of steroidogenic tissue). The TG could well be a major gonadal source of androgens in this species as such a capacity has been demonstrated in other Blenniidae (Chieffi and Botte, 1964; Seiwald and Patzner, 1987) and even in similar glands in some Gobiidae (Bonnin, 1977; Colombo et al., 1979). A measure of the TG's activity was estimated by the overall size of the gland as well as cell size, but these did not provide any clear pattern in relation to the lunar spawning cycle. However, the size of the TG did show a positive correlation to AFF size outside periods of spawning activity. This indicates that the TG

might somehow be contributing to the control of the AFF during these periods (perhaps by increasing androgen production). The fact that this correlation does not hold at peak spawning periods might suggest that the TG's role shifts during these periods. It might have a multi-functional capacity, with different functions being manifested at different times. At present, there is no clear explanation for these data.

Possible functions of the AFF :

Of the functions that have been proposed for the blenniid AFF, the two most subscribed to are that the AFF is a source of reproductive pheromones (Eggert, 1932; Losey, 1969; Laumen et al., 1974) and that it produces antibacterial secretions for egg protection (Qazim, 1956; Zander, 1975; Peterson, 1984; Heymer, 1987; Patzner, personal communication). Although the present study has clearly established the correlation between AFF development and reproduction in the species, the sampling study data do not favor one theory over the other.

Hatching of redlip blenny eggs occurs about 4.5 days after spawning (Labelle and Nursall, 1985). If the AFF produces antibacterial secretions, it might be expected to attain maximum size shortly after spawning to coincide with the incubation period. This premise, along with the observed peaks in AFF development at full moon, are consistent with Côté's (1987) findings of a peak in spawning three days

before full moon and an inferred peak in hatching one day after full moon. However, since sampling in the present study was conducted on a weekly basis, it is conceivable that the AFF size may have peaked either before or after full moon. Visual cues are apparently very important in male-male interactions (personal observation), particularly with respect to maintenance of nesting territories, and pheromones might not be involved. If male-female reproductive interactions are mediated by AFF-secreted pheromones, then a peak in AFF development before full moon would conform with a pheromonal function which would have to coincide with the time of spawning itself.

Although the morphological and histochemical studies did not address the reproductive function of the AFF, the data allow for several postulations.

Egg protection :

It is generally accepted that the function of both glandular and cuticular mucus in teleosts is protective in a general sense (Mittal et al., 1980). Various fish mucous secretions have been shown to be toxic to ectoparasites (Nigrelli, 1935), pathogenic epibionts (Nigrelli et al., 1955) and other microorganisms (Hildemann, 1962; Kitzan and Sweeney, 1968). Lysozyme and an enzyme with possible antibiotic activity have been isolated from plaice mucus (Fletcher and Grant, 1968). Immunoglobulins have been demonstrated in mucus from a number of fish species (Fletcher and Grant, 1969; Bradshaw et al., 1971). However, there is

also evidence that in spite of the active antibiotic agents present in mucus, much of its protective function is due to constant renewal (Pickering, 1974; Hattingh and Van Warmelo, 1975). Hockney (1985) was able to demonstrate that bacteria and antigens, applied to the skin as vaccines, were trapped in the mucus and subsequently cast off. Therefore, mucus from the proliferating mucous cells in the AFF at FM could provide protection to the eggs from microflora, although such a barrier would probably have to be constantly renewed.

Protection for eggs could also be provided by the proteinaceous secretions of AFF serous goblet cells. Crinotoxins, venoms and other repellent substances have been shown, in a number of fish, to be produced by various protein elaborating epidermal cells such as sacciform cells (Whitear, 1986) and other cell types resembling clavate or club cells (Cameron and Endean, 1973). Sacciform cells are distinct from serous goblet cells in that the secretion is segregated in a large membrane-bound vacuole, typically surrounded by small vacuoles or channels supplying the secretion (Whitear, 1986). Clavate cells are distinguished by having centrally placed nuclei (sometimes binucleate) and non-granular secretions. The available data on crinotoxins suggest that such secretions very likely afford protection against viruses, bacteria, fungi, algae, settling invertebrate larvae or predators (Cameron and Endean, 1973). The serous goblet cells in the redlip blenny AFF, then, could also be important in protecting the eggs by producing crinotoxins.

Pheromones :

Fish epidermal secretions have been suggested to serve a variety of pheromonal functions, including stimulating fright reactions (Von Frisch, 1941; Smith, 1982, 1985), and inducing social responses in Ictalurus spp (Todd et al., 1967; Richards, 1974). Without specifically testing the semiochemical properties of skin secretions, it has been suggested that a courtship pheromone is secreted by large mucous-like cells in the caudal gland of Corynopoma riisei (Atkins and Fink, 1979) and that reproductive pheromones are produced in the skin of male African catfish, Clarias gariepinus (Ali et al., 1987). The latter study is of special interest since it shows that the skin in Clarias is capable of synthesizing steroid glucuronides which, in turn, have been demonstrated to act as male reproductive pheromones in the same species (Lambert et al., 1986; Resink et al., 1987) as well as in the black goby, Gobius jozo (Colombo et al., 1982), and in the zebrafish, Brachydanio rerio (Van den Hurk et al., 1987).

Among Blenniidae, there are several studies on the existence of pheromones. Losey (1969) suggested that the anal fin modifications may be responsible for producing the pheromone in males of some species of Hypsoblennius which facilitates male courtship by releasing sexual appetitive behavior and by increasing sexual receptivity. Laumen et al. (1974) reported that the anal fin modifications in B. pavo produce a pheromone which attracts conspecific females. On the other hand, Peterson (1984) demonstrated that male water

from three Blennius spp had no effect on the behaviour of conspecific females, leading him to conclude that the anal fin modifications might not function in the production of pheromones.

During the current sampling study, male redlip blennies were often observed scraping and pressing their abdomens and AFFs against the substrate on their territories. While not quantified, this scraping behavior appeared to be more frequent and pronounced at FM than at other periods, and might function to deposit pheromone(s) within the territory. When captured, these males always had very swollen AFFs. Although males have been observed to engage in a behavioral display that probably advertises readiness to spawn prior to the arrival of a female on their territory (Marraro and Nursall, 1983), any pheromones deposited around the territory might assist the females to locate the territory or nest-site of a breeding male.

Proteinaceous unicellular glands typically do not open to the exterior and therefore release their products only after damage to the epidermis (Cameron and Endean, 1973). Such damage may occur during the scraping behavior as the epidermal cuticle present on the AFF in redlip blennies is generally very thin (<0.1μm). If this were true, it would be reasonable to implicate the AFF mucous goblet cells in the protection of the epidermal tissue. Nonetheless, there was little indication of any external damage in any of the AFFs examined. It is possible then, that a mucus coating on the

AFF, along with the thin cuticle could provide adequate protection from mechanical stress.

In general, where more than one type of secretory cell is present, the properties of the secretion released may depend on mixing of the products (Whitear, 1986). The secretions of the serous goblet cell might well conjugate with mucus from the mucous goblet cells as well as any secretions from the superficial epithelial cells to produce the final functional product. The proteinaceous secretions must somehow reach the exterior from the intercellular spaces into which they are apparently expelled. The final product might coat the surface of the AFF and then be deposited onto the substrate by simple physical contact. The mucus component of the secretion might either serve as a vehicle for the pheromonal components or may play a more active role as part of the actual pheromonal complex. Given the surge zone conditions which this fish inhabits, the use of pheromonal signals in reproduction would be difficult. Deposition of the semiochemical onto the substrate using a mucus base could facilitate a slow, dissipated release of the pheromonal components which could then be more easily detected in the prevalent conditions. Such chemical cues would have to be continuously replenished and this is consistent with the observation that AFF size decreases gradually after full moon.

Hence, at present, both the antibacterial and the pheromonal hypotheses could apply to the redlip blenny.

Future directions:

There is still much to learn about the AFF in O. atlanticus and in the Blenniidae in general. While there is considerable information now on the morphology of the AFF in the redlip blenny, studies are required to determine the function of the AFF. Field studies should focus on comparing the reproductive success (as assessed by number of egg batches obtained and hatched) before and after both AFFs have been removed in males. Are such surgically altered males still attractive to females and successful in maintaining their territories? A more precise sampling protocol would help to determine the exact peak of AFF development in relation to the lunar spawning cycle and whether maximum AFF development is achieved just before, during or immediately after peak spawning periods. Incidents of scraping behavior in males need to be quantified and their relationship to spawning assessed. Within the laboratory, carefully controlled behavior experiments testing the responses of conspecific females to male water, to AFF preparations and to appropriate controls are required to test the pheromonal hypothesis. The survival rate and incubation periods of laboratory-reared fertilized eggs exposed to AFF secretions could be compared with appropriate controls to test the antibacterial hypothesis.

Other questions not directly related to the reproductive function of the AFF have also been raised in the current investigation and should be addressed in future studies. The control of AFF development by hormones other than androgens

needs to be explored. Similarly, the role of the testicular gland in the development of the AFF and in reproduction in general needs to be elucidated. This could be initiated by ultrastructural and histoenzymological studies on the testicular gland cells. Ultrastructural investigations are also needed on the various components of the AFF in other blenniids. This, together with more complete biochemical analyses of secreted products would allow for more meaningful comparisons among the Blenniidae than are possible at present.

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